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The Harvard Department of Nutrition

Foreword

HARVARD'S Department of Nutrition is honored and pleased to have been invited to contribute this issue of THE AMERICAN JOURNAL OF CLINICAL NUTRITION.

This department was organized on July 1, 1942, through the vision of the late Dr. Cecil K. Drinker, then Dean and Professor of Physiology in the School of Public Health, and Dr. A. Baird Hastings, Emeritus Professor of Biological Chemistry in the Harvard Medical School. A grant from the International Health Division of the Rockefeller Foundation launched the Department of Nutrition. In addition, both the health school and the medical school contributed funds. When the five-year grant from the Rockefeller Foundation terminated, the School of Public Health increased its support of the Department substantially. In addition, generous support has been obtained from other foundations, industry (both domestic and foreign), various branches of the federal government (Public Health Service, Army, Air Force, International Cooperation Administration), and from a few private citizens. To those who have helped support our work we are most appreciative, not only for the funds and the freedom given in their use, but also for the genuine interest our various donors have shown in our work.

Like the science of nutrition and its application to current medical and health problems, the Department has grown. From an initial group of four workers the Department has increased in size and activity until for the last few years there have been some eighty workers associated with it. Each of them has played an important part in our work, particularly my colleague Dr. David Mark Hegsted, who with myself made up half of the starting team.

From the beginning we have welcomed cooperative studies not only with our colleagues at Harvard and in Boston hospitals but also with those in other universities and countries. Our first research project dealt with parenteral nutrition, specifically the preparation of fat emulsions suitable for intravenous use. This research has developed many side chains—a vehicle for administering fat-soluble carcinogens and anticarcinogens, emulsions of vitamin K_1 (now available commercially) as an antagonist for certain types of anticoagulants, and fat emulsions as a nutrient for tissue culture studies. About six months ago a fat emulsion suitable for intravenous use became available commercially. We are still working on the basic problem of parenteral nutrition, now trying to combine protein with the fat emulsion. Obesity is another problem to which the Department has devoted considerable attention for a long time. Currently our major research effort involves a number of problems related to atherosclerosis.

While most of the papers presented here properly deal with various clinical studies, we have included a few in the area of nonclinical studies to emphasize the importance of such studies to the broader field of human nutrition. We hope that the variety of these papers will illustrate some of the many ways that nutrition enters into medicine, public health, and the basic aspects of the natural sciences.

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Editorial

Nutrition, Atherosclerosis, and Infarction

One hears much these days about dietary fats as possible causative agents in atherosclerosis and, by implication, thrombosis and infarction, both coronary and cerebral. It may be well to remember that most experimental and clinical studies in this area relate to changes in the level of serum cholesterol or various lipid fractions. They do not relate directly to atherosclerosis, thrombosis, or infarction. True, in the last year or so some laboratories have included postmortem studies of actual atherosclerosis in addition to experimentally induced hypercholesteremia, and our laboratory has found a reasonable correlation between these two factors in early lesions produced in rats. One laboratory has reported the experimental production of coronary infarction in a sizable proportion of rats; nevertheless, the great mass of factual information is concerned only with changes of serum cholesterol, and these changes over a relatively short time.

Most epidemiologic studies in this area have dealt with crude death rates rather than age-specific death rates and with the information on death certificates as the cause of death. Both procedures may give erroneous results. A few epidemiologic studies have included levels of serum cholesterol, but seldom has the information on the causes of death been furnished by autopsy reports.

The level of serum cholesterol is certainly affected by the amount and kinds of fat in the diet, by a number of other components of nutrition, and by other metabolic activities such as change in body weight, relative concentration of certain hormones, exercise, and possibly by psychic stress. It is not possible to design an experiment in nutrition involving a major change in a source of calories and have only a single variable. Thus if fat is reduced in an isocaloric diet, either carbohydrate, protein, or both must increase. Defining with accuracy nutritional changes in the experimental manipulation of diets for man or changes in the usual diets of different groups of people becomes rather complicated.

Most experimental nutrition studies on raising the level of serum cholesterol and on the production of atherosclerosis involve the addition of cholesterol to the diet. At times the amount of cholesterol added to the diet has been large, as for example early studies from our laboratories where 5 per cent of the diet was cholesterol. Subsequent studies have shown that such large amounts are not necessary, and actually additions of the order of 1 mg of cholesterol per 3 to 5 calories of food consumed will result in a marked hypercholesterolemia and subsequent atherosclerosis. These studies have shown that there is both a time and an intensity factor in that the greater the level of dietary cholesterol, the shorter the time before positive results appear. Addition of cholesterol to experimental diets is a valid experimental procedure, for one is trying to produce a hypercholesteremia—and after all, man consumes a diet with a variable content of cholesterol.

With diets containing cholesterol, and more recently a small amount of cholic acid perhaps to favor absorption, it has been found that not only does the amount and type of dietary fat influence serum cholesterol (and atherosclerosis), but also the amount and type of dietary protein, carbohydrate, magnesium, and the additions of various uracil and pyrimidine compounds.

Nutrition is certainly involved (in experimental atherosclerosis), in a far more extensive and complicated way than simply through fatty acids, be they saturated, monounsaturated, or polyunsaturated. Thus, there is excellent evidence in the rat that a fat in which the predominant fatty acid is completely saturated (coconut-lauric acid) acts synergisti-

cally with a number of fats rich in the essential polyunsaturated fatty acids (linoleic and arachidonic) in providing a mixture of dietary fat that keeps serum cholesterol relatively low. Less extensive but similar data have been found also with the monkey and with man, but not with the chick.

The formula-feeding studies of a number of laboratories have shown strikingly that under the conditions of these studies fats rich in the polyunsaturated fatty acid linoleic acid, such as corn, cottonseed, or safflower oil, effect a decrease in serum cholesterol of the order of 10 to 30 per cent, depending on the initial level. These interesting studies are responsible for the wave of enthusiasm for the use of these oils in diets and emulsions of these oils as therapy. What is usually not appreciated is that these striking results were all obtained on formula diets, not on the addition of these oils to a "meat and potato" diet. On the latter there is much less evidence that a modest increase in the intake of these unsaturated oils has any effect other than to serve as an additional source of calories.

What relation does serum cholesterol have to atherosclerosis and infarction? Experimentally, the evidence is strong for atherosclerosis. The latter has now been produced in all species of animals studied when one has been able to produce a sustained elevation of serum cholesterol. It is unlikely that man would be different in this regard.

Experimentally, the evidence is weak for infarction, for only one laboratory has so far claimed to have produced infarction. It is necessary to mention that very drastic dietary conditions were necessary, and these occasionally resulted in an increase of serum cholesterol of the order of 40 times the normal. Further, infarction did not seem to be related to the level of cholesterol. Whether the milder hypercholesteremia seen in man is comparable in any way is problematical.

Evidence is also accumulating in man that an increase in serum cholesterol favors the development not only of atherosclerosis but also of infarction. However, it should be emphasized that atherosclerosis is undoubtedly a disease of multiple and additive etiology. Heredity, overweight, and hypertension are involved in addition to an increase in serum cholesterol. Further, the disease is more prevalent in smokers than in nonsmokers and in sedentary rather than physically active individuals. Since nutrition is concerned with many of these factors, it might well be a most significant factor in atherosclerosis. Its real promise would seem to lie in the area of prevention, for once one understands how nutrition is involved, opportunities for dietary changes should become available.

But it should be emphasized frequently that no discussion of nutrition and heart disease should be interpreted to mean that nutrition is the sole or perhaps even the major cause. It may be expected that the chronic diseases developing slowly over long periods will be influenced by multiple factors related to the genetic and environmental background of the patients. One of the major jobs of the researcher is to define the multiple factors involved and to determine their relative importance. The evidence today would indicate that nutrition is involved in various ways. Since the diet is subject to manipulation, there is real promise for prevention or treatment.

FREDRICK J. STARE, M.D.

Interrelations Between the Kind and Amount of Dietary Fat and Dietary Cholesterol in Experimental Hypercholesterolemia

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THE TRADITIONAL experimental approach in biologic research has been to isolate the experimental variable of interest and to study this variable independent of other factors which might influence the result. In nutrition research the use of selected inbred animals, purified diets which allow the variation of a single nutrient, constant environmental temperature control, etc., are considered as nearly a *sine qua non* of an adequate experimental design. The value of such control is clearly evident from the advances which have been made during the past.

The emphasis upon holding all variables constant except that one under study may lead, however, to the unwarranted conclusion that the variable studied has a function or action which is universal and independent of other variables. So many dietary interrelations are already known that it is obvious such assumptions can seldom be made. The point can be made, nevertheless, that often there is so much emphasis upon the nutrient under study that little consideration is given to the conditions under which the nutrient was studied.

Studies upon hypercholesterolemia and atherosclerosis, both in experimental animals and in human subjects, provide a case in point of wide interest at the present time. With human beings as subjects, it has been shown that the effects of different dietary fats upon serum cholesterol levels are (a) best correlated with iodine number or the over-all degree of unsaturation,[1] (b) explained by the amounts of saturated and polyunsaturated fatty acids in the diet, the monounsaturated fatty acids having no effect,[2] and (c) largely a reflection of the essential fatty acid or linoleic acid content.[3] Further, (d) hydrogenation of corn oil, thus decreasing both the iodine number and essential fatty acid content, has little or no influence upon its ability to lower serum cholesterol levels,[4] and (e) adding a highly saturated fat to a highly unsaturated fat does not increase the lowered serum level caused by the latter.[5] It is apparent that some of the findings are contradictory.

In experimental studies upon atherosclerosis and hypercholesterolemia the number of factors now known to be of some consequence continues to grow. In cholesterol and/or cholic acid-fed animals the following are reported to be of some importance: (a) kind and amount of fat; (b) amount of cholesterol and cholic acid; (c) kind and amount of protein; (d) kind and presumably the amount of different carbohydrates; (e) magnesium intake and possibly the amount of calcium or the calcium/

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magnesium ratio; (f) various vitamins including pyridoxine, vitamin D, ascorbic acid, and vitamin E; (g) purine and pyrimidines in the diet; (h) thyroid function; (i) sex and sex hormones; and (j) bacterial flora. This list is not all-inclusive and, of course, such variables as the length of time the experiment is run and the species used are of great importance. Certain interrelationships between some of these variables are obvious, others less so. The point which requires emphasis, however, is the distinct possibility that many and perhaps *most of these variables may have definable effects only in relation to many or all of the others.* If this should be true, then the most profitable search might not be to define the effect of fat composition on hypercholesterolemia and atherosclerosis, for example, but the interrelationships of dietary fats with other environmental and inherited factors which influence the serum cholesterol level. It is possible, perhaps likely, that the apparently contradictory studies discussed heretofore with regard to the effects of different fats on serum cholesterol levels are not contradictory but each may be true relative to the particular experimental conditions. If we assume that some or all of the variables operative in experimental animals are or might be of importance in human subjects, it is perhaps not unlikely that the formula-feeding technic using a homogeneous diet composed of fat and skim milk powder may give results quite different from those obtained with a low-fat diet of mixed foodstuffs.

The large number of variables of interest, if tested at various levels, would give an almost infinite number of combinations, outside the possibilities of a single experiment or probably of a single laboratory. Still, efforts should be made toward experimental designs which will yield the maximum amount of information with the minimum expenditure of animals, time, and effort. The present study represents a preliminary effort in this direction.

EXPERIMENTAL

The experimental design included the study of five different fats—coconut oil, olive oil, safflower oil, corn oil, and an equal mixture of coconut and safflower oil. Each of these was fed at three different levels in the diet, at 5, 10, and 20 per cent of the diet. All of these variations in kind and amount of oil were fed with 0.45 per cent cholesterol and with 1.35 per cent cholesterol in the diet. Thirty groups were thus required. Two animals per group, the minimum number necessary to obtain an estimate of variation between animals, were used. Young adult male rats weighing 250 to 300 g were housed in pairs in cages approximately 12 × 12 × 15 in. with raised screen bottoms.

The diets were similar to those previously employed.[6,7] They contained 10% casein, 5% salt mixture, 5% celluflour, 0.3% choline, 0.45% cholic acid, and vitamin supplements as previously described. The remainder of the diet consisted of the appropriate kind and amount of fat, 5, 10, or 20%, either 0.45 or 1.35% cholesterol, and sufficient glucose to complete the diet. It is recognized that the protein/calorie ratio varies in these diets depending upon the amount of fat added and that the protein intake is thus not constant. It should be noted that all diets contained cholic acid in order to produce a reasonably high serum cholesterol level.[6]

In order to minimize the time required for feeding and preparation of diets, a sufficient amount was prepared to last approximately one month and was stored in the refrigerator. The animals were fed either two or three times a week but were inspected daily to be sure that food was available. Uneaten food was discarded weekly. Such a feeding schedule results in a great saving in time as compared to the usual practice in this laboratory of feeding daily and the preparation of diets upon a weekly basis. However, it allows more exposure of the diet to the relatively high temperatures in the animal room and more opportunity for the development of rancidity or destruction of labile constituents.

Animals were bled every two weeks during the eight-week experimental period. The end of the tail was clipped and 0.2–0.3 ml of blood collected in a small centrifuge tube. This was centrifuged after clotting and 0.02 ml

TABLE I
The Experimental Design and Mean Serum Cholesterol Values (in mg/100 ml) Obtained in Each Group of Two Animals

Dietary fat		Dietary cholesterol level										
		0.45%					1.35%					
		Weeks					Weeks					
Kind	Amount %	2	4	6	8	Mean	2	4	6	8	Mean	Grand means
Clive	5	228	288	300	372	297	622	892	634	572	680	489
	10	474	544	628	472	529	492	864	804	872	758	644
	20	352	572	488	444	464	708	994	800	820	831	647
	Means	351	468	472	429	430	607	917	746	755	756	593
Coconut	5	207	402	504	445	390	328	362	422	439	388	389
	10	188	292	420	378	320	278	526	582	605	498	409
	20	148	165	286	293	223	186	190	204	191	193	208
	Means	181	286	403	372	311	264	359	403	412	359	335
Safflower	5	98	154	150	173	144	110	117	150	150	132	138
	10	148	159	190	206	176	248	198	288	290	256	216
	20	138	130	292	157	179	162	196	198	197	188	184
	Means	128	148	211	178	166	173	170	212	212	192	179
Corn	5	308	310	290	340	312	192	202	389	220	251	281
	10	260	234	304	239	259	282	208	276	324	273	266
	20	153	162	224	191	183	282	282	287	221	268	225
	Means	240	235	273	257	251	252	231	317	255	364	257
Safflower and coconut	5	273	344	302	249	292	292	216	198	206	228	260
	10	220	124	174	189	177	252	234	322	187	249	213
	20	113	180	196	166	164	184	194	208	235	205	184
	Means	202	216	224	201	211	243	215	243	209	227	219
Grand means		221	271	317	288	274	308	378	384	369	360	317

of serum taken for cholesterol analysis by a fluorimetric method.[8] A total of 240 values were thus obtained, four from each animal.

RESULTS

The mean values of each group of two animals at different times are shown in Table I. An inspection of these values will reveal some apparent effects, such as differences in the action of oils, the cholesterol level, and changes with time. Whereas some of the groups reached maximum serum cholesterol levels rather early in the experiment, others had apparently not reached maximum by the end of the eight-week period. There is an apparent difference in action of fats at different levels. For example, as the amount of olive oil or safflower oil in the diet was increased, the serum cholesterol levels rose considerably between 5 and 10 per cent fat. The further addition of olive oil had no effect, but the higher level of safflower oil may have caused a decrease in the serum cholesterol. With the safflower-coconut mixture and with corn oil, the serum cholesterol level fell with increasing amounts, and there was a considerable drop as the coconut oil was raised from 10 to 20 per cent.

The complete analysis of variance (logarithms of the serum cholesterol values were used throughout*) presented in Table II

* The general use of logarithms of the serum cholesterol values rather than the actual serum levels were preferred for several reasons. In the previously published paper[6] the serum cholesterol response was not proportional to the log of the cholesterol content of the

TABLE II
Analysis of Variance

	Degrees of freedom	Sum of squares	Mean squares	F*
Total	239	12.936896	—	—
Within groups of two	120	0.933722	—	—
Between rats	30	0.377870	0.01260	—
Within rat (time-rat interaction)	90	0.555852	0.00618	—
Treatments (between groups of two)	119	12.003174	—	—
Main effects				
Cholesterol	1	0.480932	0.48093	38.2†
Level	2	0.353932	0.17697	14.0†
Oil	4	7.267266	1.81682	144.2†
Time	3	0.506012	0.16867	27.3†
Interactions				
Chol-level	2	0.111466	0.05573	4.4‡
Chol-oil	4	0.395346	0.09884	7.8†
Chol-time	3	0.032637	0.01088	1.7
Level-oil	8	1.229425	0.15368	12.2†
Level-time	6	0.022826	0.00380	0.6
Oil-time	12	0.371844	0.03094	5.0†
Chol-level-oil	8	0.470824	0.05885	4.7†
Chol-level-time	6	0.133324	0.02222	3.6†
Chol-oil-time	12	0.086734	0.00723	1.2
Level-oil-time	24	0.245223	0.01022	1.6
Chol-level-oil-time	24	0.295383	0.01231	2.0‡

* 0.01260 was used in the denominations of those F ratios where time was not involved; 0.00618 was used in the others.
† $P < 0.01$.
‡ $0.01 < P < 0.05$.

adequately demonstrates the complexity of the situation. All of the major variables, i.e., dietary cholesterol level, level of oil, kind of oil, and time, have significant effects upon the serum cholesterol level. Many of the interactions are also of significance, indicating the difficulty of defining clearly the specific effects of any of these variables without consideration of the other variables in the experiment. It may be noted that the interactions which include time as a variable have, in general, relatively small mean squares and several were insignificant in this experiment. Thus, time was of relatively less importance than the other variables. Obviously, had two weeks not been allowed prior to the determination of the first serum cholesterol determinations, this would not have been true.

Clearly the kind of oil used was the major variable, having by far the largest mean square. The interaction of level of oil and kind of oil has the largest mean square among the interactions and would appear to be of considerable importance. Thus, it may be impossible to define an effect of an oil without consideration of the level fed. This has generally been ignored in experiments with human beings. The next largest mean square in the interactions is that of cholesterol and oil. Thus, as previously noted,[6,7] the response to cholesterol feeding is not constant when the kind of oil in the diet is varied.

While the variance analysis should provide adequate caution against undue conclusions based upon limited experiments, it is not particularly useful in attempting to define the over-all effects of oils of different kinds since it merely demonstrates that significant differences exist.

A more complete description of the experiment was attempted by computing the regression equations relating the over-all composition of the dietary oils* to the mean of the logarithms of the serum cholesterol values. For this purpose the mean of the log serum cholesterol values for each animal during the experiment (thus ignoring the effects of time and level of dietary cholesterol) were used. The amounts of saturated, monounsaturated acids, and linoleic acid provided in the different diets and the mean log serum cholesterol values are shown in Table III.

The data in Table III yield the following regression equation:

$$\text{I. log serum cholesterol} = -0.00648S + 0.02105M - 0.02415P + 2.4836$$

where S, M, and P equal grams of saturated,

diet when different oils were included in the diet. It has since been shown that the response curves with different oils are essentially parallel if log dose–log serum cholesterol is plotted. The comparison at different levels of cholesterol feeding is thus greatly facilitated. It is also known that the technical error in cholesterol measurement is increased as the serum cholesterol level rises (Watkins *et al.*, *J. Clin. Investigation* 33: 874, 1954). Statistical comparison is more appropriately made when this variation is minimized by the use of logarithms.

* The oils and the analytical results were kindly supplied through the courtesy of Dr. Fred Mattson, Procter and Gamble, Cincinnati, Ohio.

TABLE III
Diet Composition and the Mean of the Logs of the Serum Cholesterol Values

Kind of oil	Amount %	Fatty acid content, %			Mean serum log cholesterol	
		Sat'd	Mono	Poly	Found	Calculated*
Olive	5	0.56	4.20	0.25	2.6874 2.6088	2.5623
	10	1.22	8.40	0.49	2.7917 2.7879	2.6407
	20	2.44	16.80	0.98	2.7754 2.7970	2.7977
Coconut	5	4.52	0.38	0.10	2.5587 2.5909	2.4599
	10	9.03	0.76	0.20	2.5703 2.5652	2.4362
	20	18.10	1.52	0.40	2.3252 2.2839	2.3885
Safflower	5	0.59	0.31	4.05	2.1088 2.1312	2.3885
	10	1.17	0.61	8.10	2.2709 2.3626	2.2932
	20	2.34	1.22	16.20	2.2462 2.2532	2.1029
Corn	5	0.59	2.32	2.10	2.4561 2.4134	2.4779
	10	1.17	4.63	4.20	2.4503 2.3860	2.4720
	20	2.35	9.26	8.40	2.3028 2.3645	2.4604
Coconut and safflower	5	2.55	0.35	2.08	2.4126 2.3972	2.4242
	10	5.10	0.69	4.16	2.2867 2.3296	2.3646
	20	10.20	1.38	8.32	2.2756 2.2384	2.2455

* According to equation I (see text).

monounsaturated, and polyunsaturated acid per 100 g of diet, respectively. The coefficient of multiple correlation, R, is 0.811. According to this equation, the polyunsaturated acid (in this case linoleic acid, since the oils contained insignificant amounts of other polyunsaturated acids) is primarily responsible for the lowering of the serum cholesterol, having a coefficient of -0.02415. The saturated acids contribute in the same direction but appear to have only about one-fourth the activity. The monounsaturated acids, on the other

hand, gave a positive coefficient of 0.02105. As the amount of oleic acid increased the serum cholesterol value rose. Thus, linoleic and oleic acids apparently had about equal and opposite effects.

In the above analysis the influence of time and cholesterol level in the diet were ignored. In order to examine partially the effects of time and cholesterol level, the regression equations were calculated for the two cholesterol levels separately over the entire experiment and also for the data on the fourth week alone. The regression coefficients and multiple correlation coefficients are shown in Table IV. The

TABLE IV

Regression Coefficients Relating Oil Composition to Log Serum Cholesterol Level at Different Times and Cholesterol Intake

Data included	Regression coefficients			R
	S	M	P	
Both cholesterol, all weeks	−0.00648	+0.02105	−0.02415	0.807
1.35% cholesterol, all weeks	−0.00668	+0.02855	−0.02365	0.774
0.45% cholesterol, all weeks	−0.00618	+0.01428	−0.02443	0.772
1.35% cholesterol, 4th week only	−0.0045	+0.0352	−0.0946	0.753
0.45% cholesterol, 4th week only	−0.0091	+0.0286	−0.0210	0.644

magnitude of the regression coefficients varies considerably with the selected data, as might be expected from the previously demonstrated interactions of time, oil, level of oil, and level of cholesterol. The sign of the regression coefficients remains the same, however, and, as might be expected, the coefficient of multiple regression decreases with more limited data.

Plots of the calculated log serum cholesterol versus the actual values found are of some interest. In Figure 1, the actual values and those calculated from equation I are shown. It is apparent that the fit is reasonably good but that the equation underestimates the effect of the 5 per cent safflower oil diet and overestimates the effect of the 20 per cent safflower diet. All the equations in Table II fail to adequately place some of the oils,

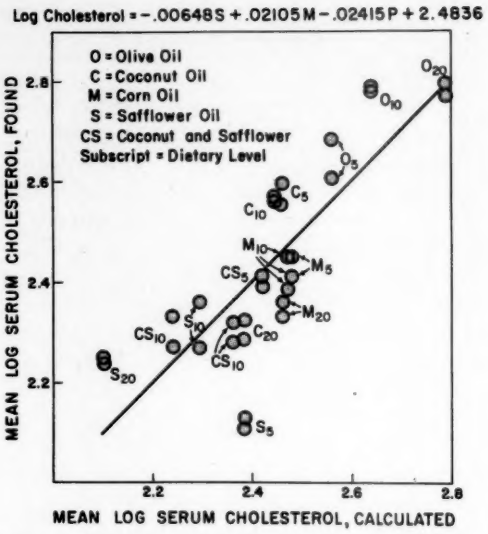

Fig. 1. The relationship between the calculated mean log serum cholesterol values according to the equation and the values from which the equation was derived.

usually the 5 or 20 per cent safflower group or both.

This suggested that the inclusion of the total level of oil in the equation might improve the fit. Thus, the following equation was derived:

$$\text{II.}'\ \text{mean log chol.} = +0.00531S + 0.03268M - 0.01128P - 0.01160\%\ \text{fat} + 2.4778$$

This equation fails to improve the fit to any appreciable degree over that obtained with equation I. It is instructive to note the changes in the coefficients of S, M, and P when the fourth variable is included in the equation. The coefficient of S is of approximately the same size as in equation I but opposite in sign. The coefficient of M is about 50 per cent larger while the coefficient of P is only half as large. As Snedecor[9] has pointed out, a general feature of multiple regression is that the various coefficients are intercorrelated and the introduction of a new variable changes all of the other coefficients. Thus, it is difficult to evaluate the absolute meaning of any of the coefficients, since in any equation the size and perhaps even the sign may be changed if another variable is included or omitted.

In a previous publication[7] we concluded that when diets were fed containing a constant amount of fat of different kinds, the product of the saturated and linoleic acid content of the diet was a parameter with a very high negative correlation with the log of serum cholesterol level ($r = -0.94$). Hence, for these data the equation relating the product and amount of oil was calculated. This yields:

III. log mean serum chol. = 0.0171% fat − 0.274 log product + 2.6745

The coefficient of multiple regression is 0.80. As seen from the plot of the calculated versus actual values (Fig. 2) the fit is about the

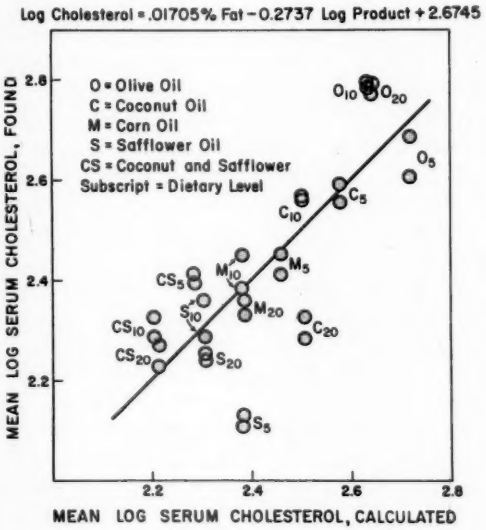

Fig. 2. The calculated mean log serum cholesterol values obtained from the equation are plotted against the actually determined values.

same as in the previous plots of equation I Here the 5 per cent safflower diet and the 20 per cent coconut diet are the most seriously misrepresented by the equation. This equation has a positive coefficient for the percentage of fat in the diet whereas in equation II including this variable gave a negative coefficient. Again the danger of attaching too much physical significance to the regression coefficient is apparent.

SUMMARY

An extremely large number of variables are reported to influence hypercholesterolemia and atherosclerosis in experimental studies, and there is need for experimental designs adequate to evaluate the relative importance of these variables and their interrelation to each other. A study is reported in which five different oils, each at three levels, and two different levels of dietary cholesterol were investigated. Only two animals per groups were used. All of the major variables (time, kinds of oil, level of oil, and amount of dietary cholesterol) have significant effects upon the hypercholesterolemia and many of the numerous interactions are also significant. It would appear, therefore, that the action of any of these variables can only be stated at the present time in terms relative to the others. The same situation is probably true with other variables not specifically included in this study. Such interrelations may well account for the apparently contradictory results in the literature as to the effects of various fats upon hypercholesterolemia in human subjects.

Regression equations relating the amount of fatty acid (saturated, monounsaturated, and polyunsaturated) in the diet to the serum cholesterol level were calculated. With these three variables in the equation, the coefficient for the monounsaturated acid is positive. The coefficients for the saturated and polyunsaturated acids are negative.

The equation suggests that the monounsaturated acid raises the serum cholesterol level while the saturated and polyunsaturated acids reduce it, the saturated acids being about one-fourth as active as the polyunsaturated acid. However, other equations can be derived which give as good a "fit" as this equation, and the magnitude and even the sign of the coefficients vary depending upon the variables included in the equation. Caution should be used in assigning too much significance to the calculated coefficients.

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Studies on the Interrelationships Between Dietary Magnesium and Calcium in Atherogenesis and Renal Lesions

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RECENT studies from this laboratory demonstrated that the amount of lipid deposited in the left ventricular valves and aorta of 21-day-old rats fed a diet containing cholesterol and cholic acid could be markedly reduced by feeding large quantities of magnesium.[1,2] This atherogenic diet increased the dietary requirement for magnesium as evidenced by decreased rates of growth and decreased oxidative phosphorylation by heart mitochondrial preparations.[1] Various investigators have presented evidence that at low levels of dietary magnesium, increased dietary calcium levels aggravated the signs and symptoms of magnesium deficiency and decreased the rates of growth.[3,4] Studies on the interrelationships between dietary calcium and magnesium need not be reviewed here except to indicate that one of the principal effects of magnesium deficiency is to produce calcification of various soft tissues, including the heart and aorta.[5,6,7] A major part of the present investigation was to determine whether low, medium, and high levels of dietary calcium increased the severity of the vascular sudanophilia seen in animals fed low and high magnesium diets.

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METHODS

The basic diet consisted of the following per 100 g of diet: casein (purified), 10; glucose, 58.4; fat (Spry), 20; celluflour, 5.0; Jones-Foster[8] salt mixture with $CaCO_3$ and $MgSO_4$ removed, 5.0; and $CaCO_3$, 1.5. Fat-soluble and water-soluble vitamins were also added.[1] The variation between the groups of animals lay in (a) the amount of dietary magnesium, as MgO, 24 and 192 mg per 100 g: (b) the presence or absence of cholesterol (1.0 per cent) and/or cholic acid (0.3 per cent); and (c) the amount of dietary calcium, as $CaCO_3$, 200, 600, or 1200 mg per 100 g. The cholesterol and/or cholic acid and the calcium were added at the expense of glucose.

Twenty-one-day-old male rats obtained from the Charles River Breeding Laboratories, Inc., Boston, Mass., were housed in individual cages and fed the various diets ad libitum for a period of approximately 28 days. At the end of each experiment the animals were sacrificed by decapitation and blood collected for the purpose of determining serum cholesterol[9] and serum proteins and lipoproteins by paper electrophoresis.[10] Sections of liver, kidney, and heart were fixed in 10 per cent formalin for microscopic examination. After the heart and aorta had been opened to expose the left ventricular valves and aorta and fixed in formalin, they were stained with Sudan IV and graded as to the extent of sudanophilia (heart score) as previously described.[1] In several of the experiments, the left kidney of each animal

was decapsulated and weighed. The extent of the microscopic calcium deposition in tubular lumens, mainly in the zona intermedia, the outer portion of the medulla of the kidney, was scored on the basis of 0 to plus 4 (kidney score).[2]

RESULTS

Effect of Dietary Cholesterol and/or Cholic Acid on Renal Calcium Deposition

Table I demonstrates the separate and combined effects of dietary cholesterol and cholic acid on atherogenesis, kidney score, and serum cholesterol levels at two levels of magnesium intake. The heart score for animals fed both cholesterol and cholic acid was 7.6 at magnesium intakes of 24 mg per 100 g of diet. This was reduced to 5.5 when the level of dietary magnesium was increased and the kidney lesions were nearly abolished. There was, however, no difference in the serum cholesterol level or in the rate of weight gain of the two groups. The addition of cholesterol alone to the basic diet containing low magnesium resulted in a serum cholesterol level of 200 mg per 100 ml compared to approximately 700 mg per 100 ml when both cholesterol and cholic acid were added. The heart score was 2.9 and this was further decreased to 0.8 by increasing the dietary magnesium to 192 mg per 100 g. As before, the level of dietary magnesium had no effect upon the serum cholesterol level. The kidney score of 1.8 was the same as when the diet contained both cholesterol and cholic acid. Increasing the level of dietary magnesium again practically prevented the development of kidney lesions. The addition of only cholic acid to diet caused only a slight hypercholesterolemia. The heart score of 2 was apparently not affected by the level of dietary magnesium. It appears that either dietary cholesterol or cholic acid results in kidney changes and the effects are not additive or related to the serum cholesterol level.

TABLE I

Effect of Dietary Cholesterol (1%) and/or Cholic Acid (0.3%) on Vascular Sudanophilia and Renal Calcium Deposition

Dietary* (34 days)						
Cholesterol (1 g %)	Cholic acid (0.3 g %)	Mg (mg %)	Weight gain (g)	Serum cholesterol (mg %)	Heart score†	Kidney score†
+	+	24	60 (8)‡	720	7.6	1.8
+	+	192	63 (9)	704	5.5	0-Trace
+	0	24	78 (7)	227	2.9	1.8
+	0	192	92 (7)	192	0.8	0-Trace
0	+	24	46 (8)	122	1.6	1.2
0	+	192	54 (7)	134	2.3	0-Trace

* Calcium was added at a level of 600 mg per 100 g to all diets.

† Heart score represents the amount of lipid deposited in the left ventricular valves and aorta seen under a dissecting microscope (×10) and kidney score represents the amount of calcium deposited in the renal tubular lumens seen microscopically.

‡ Number of animals per group.

On the average the animals fed the high dietary magnesium diets gained more weight than those fed the low-magnesium diets. Those fed only cholesterol gained the most weight while those fed the diet containing only cholic acid grew the least. The animals fed the diet containing both cholesterol and cholic acid grew slightly faster than those fed only cholic acid.

Effect of Dietary Magnesium and Calcium on Heart and Kidney Score, Serum Cholesterol, and Lipoproteins

Table II illustrates the results of a similar study in which two levels of dietary calcium were also studied. In general, the addition of magnesium resulted in increased weight gains at either high or medium calcium intakes. This was true whether cholesterol and/or cholic acid were added to the diet. The addition of only cholic acid to the various diets resulted in the poorest weight gains, whereas the addition of cholesterol usually resulted in somewhat greater weight gains than those in animals fed no cholesterol and no cholic acid.

The addition of only cholic acid to the basic diet resulted in serum cholesterol levels ranging from 142 to 154 mg per 100 ml regardless of the level of dietary magnesium or calcium fed. Control animals had serum cholesterol levels of approximately 115 mg per 100 ml.

The serum cholesterol level of animals fed

TABLE II

Effect of Dietary Magnesium and Calcium on Heart and Kidney Score, Serum Cholesterol, and Lipoproteins

Dietary (24 days)				Weight gain* (g)	Serum cholesterol (mg %)	Heart score	Kidney score	Lipoproteins† (sq cm)	
Cholesterol (1.0 g %)	Cholic acid (0.3 g %)	Mg (mg %)	Ca (mg %)					Alpha	Beta
−	−	24	600	41	108	0.0	0.9	7.3	10.0
+	−	24	600	44	261	1.8	0.3	5.4	19.9
−	+	24	600	36	148	0.7	0.7	5.7	11.1
+	+	24	600	35	515	4.7	1.3	3.5	26.1
−	−	192	600	56	114	0.0	0	6.6	6.1
+	−	192	600	52	256	0.7	to	4.4	15.5
−	+	192	600	32	154	1.1	trace	6.8	11.1
+	+	192	600	39	705	3.0		2.6	23.5
−	−	24	1200	35	117	0.0	0.3	2.4	4.7
+	−	24	1200	41	191	1.0	1.5	2.0	8.9
−	+	24	1200	25	148	1.1	1.0	5.0	8.3
+	+	24	1200	28	748	8.3	1.2	2.1	35.9
−	−	192	1200	47	115	0.0	0	3.8	5.7
+	−	192	1200	51	136	0.9	to	6.7	10.1
−	+	192	1200	34	142	0.9	trace	7.0	10.8
+	+	192	1200	34	818	4.0		3.7	29.7

* Six animals per group.

† These values represent the total stainable lipid of the alpha and beta lipoproteins determined by paper electrophoresis.

only cholesterol and 600 mg per 100 g calcium, regardless of the magnesium levels, was approximately 260 mg per 100 ml. Increasing the calcium level to 1200 mg per 100 g significantly reduced the serum cholesterol level to 191 and 136 mg per 100 ml at the two levels of magnesium. Increasing the dietary calcium caused some decrease in the serum cholesterol of the animals fed cholesterol alone. However, when both cholesterol and cholic acid were fed, increasing the level of either dietary magnesium or calcium resulted in an increase in the serum cholesterol level.

The addition of cholesterol or cholic acid singly resulted in slight vascular sudanophilia which was not influenced greatly by either the magnesium or calcium intakes. The combination of both steroids to the basic diet resulted in significant amounts of lipid deposit in the heart valves and aorta. At either level of calcium intake, high magnesium diets resulted in decreased heart scores. However, increasing the level of calcium from 600 to 1200 mg per 100 g at 24 mg per 100 g magnesium resulted in a significant increase in heart score from 4.7 to 8.3.

All animals consuming diets containing 24 mg magnesium and 600 mg calcium per 100 g showed renal damage of approximately the same magnitude. The low kidney score, 0.3, of the animals whose diets contained cholic acid could be misleading, for it reflects only the degree of calcium deposition. The fairly extensive tubular dilatation in these animals is not reflected in this low score. The high-calcium diet seemed to have afforded some renal protection to the animals fed the cholesterol–cholic acid-free and low-magnesium diets. The addition of either cholesterol or cholic acid resulted in the same degree of renal calcification as did the addition of both steroids. Regardless of the level of dietary calcium or whether cholesterol and/or cholic acid were included in the diet, the feeding of 192 mg per 100 g magnesium diets resulted in kidney calcium deposition scores of 0 to trace.

Animals fed the high-calcium and cholesterol–cholic acid-free diets tended to have low levels of both alpha and beta lipoproteins which were not influenced by the level of dietary magnesium. At the lower level of calcium intake (600 mg per 100 g) high dietary

magnesium resulted in a decrease in both the alpha and beta lipoproteins, although not to the same level as when high calcium diets were fed. The addition of cholesterol and cholic acid to the diet resulted in significant increases in the beta fraction which tended to be higher in the high calcium-fed groups and not influenced by the level of dietary magnesium.

Effect of Dietary Magnesium and Calcium in Cholesterol–Cholic Acid-Fed Rats

A portion of the previous experiment was repeated and the results are seen in Table III in which a third level of calcium was studied.

TABLE III

Effect of Dietary Magnesium and Calcium on Vascular Sudanophilia and Renal Calcium Deposition in Cholesterol (1%) and Cholic Acid (0.3%) Fed Rats

Dietary (24 days)		Weight gain (g)	Serum cholesterol (mg %)	Kidney score	Heart score
Mg (mg %)	Ca (mg %)				
24 (6)*	200	44	589	0.8	4.7
24 (6)	600	33	621	2.1	6.0
24 (6)	1200	31	775	2.0	4.8
192 (6)	200	28	938	0–Trace	5.1
192 (6)	600	45	776	0–Trace	1.5
192 (6)	1200	62	656	0–Trace	2.1

* Number of animals per group.

At low levels of magnesium (24 mg per 100 ml) increasing the level of calcium from 200 to 1200 mg per 100 ml resulted in less weight gain over the 24-day test period. The reverse was true at high levels of magnesium. Increasing the level of calcium from 200 to 1200 mg per 100 g resulted in increased weight gains from 28 to 62 g. This was not seen in the previous experiment (Table II) in which calcium was without apparent effect on the rate of weight gain in cholesterol–cholic acid-fed animals.

Unlike the changes in weight gain, increasing the level of calcium from low to high resulted in a significant rise in serum cholesterol levels in animals fed the low-magnesium diet. The reverse was true at high levels of magnesium.

As in the previous experiments, high dietary magnesium markedly decreased or abolished the kidney lesion or renal tubular calcification, regardless of the level of calcium fed. The low-calcium diet (200 mg per 100 g) afforded greater protection against renal tubular calcification than that seen in animals fed either the higher calcium diets (600 or 1200 mg per 100 ml).

In this study the effect of high dietary magnesium in lowering the heart score was observed only when the dietary calcium was above 200 mg per 100 g.

Effect of Dietary Calcium and Magnesium on Serum Cholesterol and Heart and Kidney Score

Table IV illustrates the results of another experiment, similar to that reported in Table III but with additional groups of animals who were fed cholesterol–cholic acid-free diets.

In animals fed cholesterol–cholic acid-free diets, decreasing the level of dietary calcium from 1200 or 600 to 200 mg per 100 g at low magnesium intakes (24 mg per 100 g) had no effect upon weight gain but significantly increased the level of serum cholesterol and decreased the kidney weight and kidney-body weight ratio. There was no apparent effect on the amount of renal calcification seen microscopically (kidney score). At the high level of dietary magnesium (192 mg per 100 g), increased levels of calcium resulted in increased weight gain and decreased levels of serum cholesterol. High dietary magnesium again abolished renal tubular calcification regardless of the level of calcium fed. The kidney weights and kidney-body weight ratios were essentially the same for all groups. No lipid was seen in the heart valves or aortas of animals fed cholesterol–cholic acid-free diets.

The results were considerably different when the diets contained cholesterol and cholic acid. At the low level of dietary magnesium, increasing the level of dietary calcium from 200 to 1200 mg per 100 g tended to decrease the rate of weight gain, whereas the level of dietary calcium in animals fed the high level of magnesium was without effect on the rate of weight gain. The dietary calcium level was without a clear effect on serum cholesterol in the groups fed the low-magnesium diets, but it decreased the serum cholesterol level in animals fed the high-magnesium diet. At

TABLE IV

Effect of Dietary Calcium and Magnesium on Serum Cholesterol and Heart and Kidney Score

Dietary (24 days)		Weight gain* (g)	Serum cholesterol (mg %)	Kidney†			Heart score
Mg (mg %)	Ca (mg %)			Weight (mg)	K.W./B.W. (ratio)	Score	
WITHOUT DIETARY CHOLESTEROL AND CHOLIC ACID							
24	200	57	265	509	.44	1.2	—
24	600	53	124	743	.58	1.5	—
24	1200	61	106	738	.58	1.2	—
192	200	51	205	509	.41	0	—
192	600	63	101	490	.38	to	—
192	1200	76	96	519	.38	trace	—
WITH DIETARY CHOLESTEROL (1%) AND CHOLIC ACID (0.3%) ADDED							
24	200	36	796	530	.51	1.2	4.1
24	600	31	652	741	.79	1.4	3.2
24	1200	29	856	579	.57	1.0	3.4
192	200	45	792	483	.41	0	4.1
192	600	48	773	446	.39	to	1.6
192	1200	42	544	458	.42	trace	2.1

* Ten animals per group.

† Left kidney divided by body weight.

TABLE V

Effect of Dietary Calcium and Magnesium on Protein and Lipoprotein Fractions

Dietary (24 days)		Serum proteins (g %)					Serum lipoproteins (sq cm)	
Mg (mg %)	Ca (mg %)	Albumin	α_1	α_2	β	γ	α	β
WITHOUT ADDED DIETARY CHOLESTEROL AND CHOLIC ACID								
24	200	2.72	0.98	0.48	1.05	0.46	6.4	30.0
24	600	2.88	0.79	0.36	1.00	0.36	9.3	12.5
24	1200	2.59	0.78	0.47	0.96	0.43	11.5	23.0
192	200	2.63	0.82	0.47	1.01	0.43	2.6	20.1
192	600	2.83	0.75	0.45	0.98	0.54	7.6	6.8
192	1200	2.82	0.76	0.41	0.81	0.43	8.4	10.0
WITH ADDED DIETARY CHOLESTEROL (1.0%) AND CHOLIC ACID (0.3%)								
24	200	2.45	0.95	0.41	1.15	0.48	4.4	51.5
24	600	2.39	1.01	0.49	1.25	0.58	1.0	27.9
24	1200	2.41	0.92	0.41	1.25	0.43	1.5	45.4
192	200	2.32	0.93	0.44	1.02	0.48	1.9	38.6
192	600	2.28	0.89	0.46	1.14	0.38	1.2	48.1
192	1200	2.41	0.85	0.43	1.07	0.57	0.5	43.1

low magnesium intakes, low-calcium diets resulted in a slightly smaller kidney and a reduced kidney-body weight ratio compared to animals fed the higher levels of calcium. With or without cholesterol-cholic acid added to the diet, there was some protection by low-calcium diets on the kidney profile, although this was not true when the kidney was evaluated microscopically for calcification (kidney score). The protective effect of high dietary magnesium on the kidney profile was observed. There was no apparent effect of calcium on the heart

score of animals fed the low-magnesium diets. The expected decrease in the heart score of animals fed high-magnesium diets was again not observed in the group fed the low-calcium diet but was observed in the groups fed the 600 or 1200 mg per 100 g calcium diets.

Effect of Dietary Calcium and Magnesium on Protein and Lipoprotein Fractions

Table V lists the serum protein and lipoprotein fractions of the animals fed the diets listed in Table IV. In animals fed cholesterol and cholic acid-free diets, the alpha and beta lipoproteins were reduced at any level of calcium by increasing dietary magnesium from 24 to 192 mg per 100 g. At both these levels of magnesium, increasing the level of calcium from 200 to 1200 mg per 100 g resulted in increased alpha lipoproteins. Although the beta lipoproteins were also decreased by increased dietary magnesium at any given level of calcium, the lowest area found for this fraction was when diets containing 600 mg calcium per 100 g were fed. Cholesterol and cholic acid feeding resulted in lowered alpha and increased beta lipoproteins in contrast to animals fed diets free of these steroids. There was no consistent effect of either dietary magnesium or calcium on these fractions, with the possible exception of the alpha lipoproteins, in these cholesterol-cholic acid-fed animals. Increasing the level of calcium tended to cause a decrease in alpha lipoproteins in contrast to animals fed diets free of these steroids. Increased dietary magnesium resulted in some lowering of the alpha lipoproteins and was without effect on the beta lipoproteins.

The total serum protein was not effected by either dietary magnesium, calcium, or dietary cholesterol and cholic acid. However, the albumin fraction was consistently lower, while the alpha and beta globulins were consistently higher, in animals fed cholesterol and cholic acid.

DISCUSSION

As has been emphasized elsewhere,[11] the number of variables which may influence the development of hypercholesterolemia and the response of the animal or tissues to this condition is continually increasing as research continues. One of the major purposes of experimental work is to define the relative importance of these variables and their interactions. In this study the calcium and magnesium levels as well as the presence or absence of cholesterol and cholic acid in the diet have been investigated. Growth, serum cholesterol and proteins, the extent of vascular sudanophilia, and kidney lesions have been appraised. It may be expected on the basis

TABLE VI

Summary of Effects Observed at High vs. Low Dietary Magnesium*

Table containing original data	Dietary Ca levels (mg %)	Growth	Serum cholesterol	Heart score	Kidney score
CHOLESTEROL–CHOLATE FED ANIMALS					
I	600	0*	0*	↓*	↓*
II	600	0	↑	↓	↓
	1200	?↑	↑	↓	↓
III	200	↓	↑	0	↓
	600	↑	?↑	↓	↓
	1200	↑	?↓	↓	↓
IV	200	?↑	0	0	↓
	600	↑	?↑	↓	↓
	1200	↑	↓	↓	↓
CHOLESTEROL ONLY FED					
I	600	↑	0	↓	↓
II	600	?↑	0	↓	↓
	1200	?↑	?↓	0	↓
CHOLIC ACID ONLY FED					
I	600	?↑	0†	?↑	↓
II	600	?↓	0†	0	↓
	1200	↑	0†	0	↓
NO CHOLESTEROL OR CHOLIC ACID FED					
II	600	↑	0†	0‡	↓
	1200	↑	0†	0‡	↓
IV	200	?↓	?↓	0‡	↓
	600	?↑	?↓	0‡	↓
	1200	↑	0†	0‡	↓

* The symbols ↓, ?↓, 0, ?↑, ↑, indicate a definite decrease, a questionable decrease, no change, a questionable increase, or a definite increase, respectively, when the 192 mg % magnesium diet group was compared to the group on the same diet but contained only 24 mg % magnesium.

† Serum cholesterol only slightly elevated in either group.

‡ No heart score observed in either group.

TABLE VII

Summary of Effects Observed at Different Levels of Dietary Calcium*

Table containing original data	Ca levels compared (mg %)	Dietary Mg level (mg %)	Growth	Serum cholesterol	Heart score	Kidney score
			CHOLESTEROL–CHOLATE FED ANIMALS			
II	600 vs 1200	24	↓*	↑*	↑*	0*
	600 vs 1200	192	0	?↑	?↑	0†
III	200 vs 600	24	↓	↑	?↑	↑
	600 vs 1200	24	0	?↑	0	0
	200 vs 600	192	↑	↓	↓	0†
	600 vs 1200	192	↑	?↓	0	0†
IV	200 vs 600	24	?↓	?↓	0	0
	600 vs 1200	24	0	?↑	0	0
	200 vs 600	192	0	0	↓	0†
	600 vs 1200	192	?↓	?↓	0	0†
			CHOLESTEROL ONLY FED			
II	600 vs 1200	24	0	↓	?↑	?↑
	600 vs 1200	192	0	↓	0	0†
			CHOLIC ACID ONLY FED			
II	600 vs 1200	24	↓	0	?↑	0
	600 vs 1200	192	0	0	0	0†
			NO CHOLESTEROL OR CHOLIC ACID			
II	600 vs 1200	24	?↓	0	0‡	↓
	600 vs 1200	192	↓	0	0‡	0†
IV	200 vs 600	24	0	↓	0‡	0
	600 vs 1200	24	↑	?↓	0‡	0
	200 vs 600	192	↑	↓	0‡	0†
	600 vs 1200	192	↑	0	0‡	0†

* In all cases the symbols indicate the result obtained at the higher level of calcium compared to the lower. The symbols, ↓, ?↓, 0, ?↑, ↑, indicate a definite decrease, a questionable decrease, no change, questionable increase, and a definite increase, respectively. Thus, in the first line the animals receiving the higher level of calcium (1200 mg %), grew less well, had higher serum cholesterol levels, higher heart scores, and similar kidney scores as compared to the animals receiving the lower calcium level (600 mg %).

† The higher level of magnesium (192 mg %) was effective in preventing the occurrence of calcification in the kidney regardless of the level of dietary calcium.

‡ The serum cholesterol was only slightly elevated if at all in these animals and no appreciable amount of vascular sudanophilia was observed.

of past experience that effects of these dietary variables are dependent upon their concentrations and upon the lesions produced. For example, the presence or absence of kidney lesions might affect the rate of growth or influence the effect of dietary calcium upon growth. Changes in growth rates, caused either by the experimental conditions or by the nature of the animals used, may affect the response of serum lipids.

In an attempt to facilitate comparisons of the results of different experiments, Table VI was prepared, which indicates the responses obtained when the dietary magnesium was raised from 24 mg to 192 mg per 100 g, other factors being held constant. Table VII is comparable to Table VI in that it demonstrates the effect of changing the concentration of dietary calcium. It is clear that additional magnesium always decreases or eliminates calcium deposition in the kidney regardless of other dietary variables. It may be concluded that this

lesion is due to magnesium deficiency. Adding cholesterol and cholic acid to low-magnesium diets was not consistent in increasing the severity of renal tubular calcium deposition. Possibly, this lesion develops at different rates, and thus different experimental durations or levels of dietary magnesium would be needed to demonstrate the effect of the cholesterol–cholic acid diet.

Other parameters in evaluating the response of the kidney to the diets are probably needed. The degree of calcium deposition in the tubular lumens is an inadequate criterion, for it alone does not always adequately reflect even the degree of morphologic damage. Instead of "grading" renal tubular dilatation (which was of lesser magnitude in the 24 mg per 100 g magnesium groups with 200 mg per 100 g calcium than with higher calcium), it would seem reasonable to use kidney weight and the ratio of kidney weight to body weight, these being more objective and numerically more precise. The data in Table IV suggest that the kidneys of animals fed 192 mg per 100 g magnesium, kidneys which show little or no morphologic damage, have a fairly set ratio to body weight rather than to age. Thus, the increased ratio of kidney weight to body weight is probably an indication of renal abnormality. Quantitative chemical analyses of renal calcium and more particularly studies at the functional level may be useful.

In the cholesterol–cholic acid-fed animals (with marked hypercholesterolemia), magnesium was always effective in lowering the heart score with one apparently clear exception, i.e., when the dietary calcium was at the lowest level fed (200 mg per 100 g). Thus, at least a minimum level of dietary calcium must be fed before this action of magnesium is manifested. In animals fed only cholesterol or cholic acid and with much lower levels of serum cholesterol, the heart scores were relatively low and the action of magnesium less clear-cut. At least in the cholesterol-fed animals it probably acts to decrease the extent of the lesions.

The effect of dietary calcium upon changes in the heart score, if any, is not clear. An inspection of Table VII suggests that the changes observed are possibly consistent with the changes seen in the serum cholesterol levels and thus may be the result of differences in serum cholesterol levels. At least, at the present time, there appears to be no reason to postulate an effect of calcium upon the development of vascular sudanophilia, independent of the serum cholesterol level. The marked difference between the effect of calcium and the effect of magnesium is clear, since additional magnesium decreased heart score consistently in the face of constant or increasing serum cholesterol levels.

We have previously reported that dietary magnesium levels have little effect upon serum cholesterol levels but that the serum cholesterol generally tended to rise as the dietary magnesium was raised. The present data are also equivocal as to whether magnesium has any direct effect upon the serum cholesterol level. It may be assumed to be relatively unimportant in this regard. Similarly, in the cholesterol–cholic acid-fed animals with marked hypercholesterolemia, the effects of calcium were minor or equivocal. It may be noted that in the cholesterol-fed animals (Table II), the high calcium levels resulted in a considerable decrease in the serum cholesterol level. Consistent trends were also noted in the animals fed no cholesterol or cholic acid. It should be noted, particularly, that the serum cholesterol values—above 200 mg per 100 ml observed in the animals fed the low-calcium diet—are unusually high. Thus, calcium may be of some importance under certain circumstances.

On the basis of growth experiments the conclusion was reached in earlier work[3] that the minimum dietary requirement of the young rat for magnesium was approximately 25 mg per 100 g. The present data upon kidney lesions demonstrate that this estimate is too low. The impression is gained from these experiments that additional magnesium generally stimulates growth to some extent. However, when dietary calcium was low (200 mg per 100 g) additional magnesium may be detrimental (Table III). The effects of calcium upon growth are also probably dependent upon the level of dietary magnesium and whether cholesterol and/or cholic acid was fed. In general, it appears that high levels of calcium

usually decrease the rate of growth if the magnesium is at the minimal level of 24 mg per 100 g. This is the result that might be expected, according to the data in literature indicating adverse effects of calcium on low-magnesium diets. On the other hand, when the level of dietary magnesium is high and not a growth determinant, additional calcium may often stimulate growth to some extent.

In studies of this kind the levels of nutrients to be investigated must be chosen more or less arbitrarily. If the level chosen for any particular diet is critical, i.e., only approaching the level of adequacy, for any particular diet, then minor changes in other constituents of the diet or the condition of the animal may change its relative importance in one experiment to another. This may be the situation with respect to the low levels of magnesium and calcium which were chosen in these studies.

The somewhat lower serum albumin levels in the cholesterol–cholic acid-fed animals (Table V) may be largely related to hepatic damage. They cannot be explained on the basis of renal damage seen morphologically, since the low-magnesium animals without cholesterol and cholic acid had equally damaged kidneys (kidney score) but higher albumin levels. Also, the addition of magnesium to the cholesterol–cholic acid-containing diet eliminated the kidney calcification without affecting the albumin level. Cholesterol–cholic acid feeding produces significant amounts of lipid and cholesterol deposit in and enlargement of the liver. On the other hand, the animals fed the low-calcium (200 mg per 100 g), low-magnesium (24 mg per 100 g), and cholesterol–cholic acid-free diets had essentially normal serum albumin levels despite the fact that they had gross fatty livers with some cholesterol deposition.

The mechanisms by which magnesium reduces vascular sudanophilia remain obscure. However, in the limited studies on the alpha and beta lipoproteins presented here (Table V), as well as in other studies,[12] the animals fed the low-magnesium diet without added cholesterol and cholic acid showed increased concentrations of both alpha and beta lipoproteins. Additional dietary magnesium lowered these at each level of calcium that was studied. Although these effects are not readily seen in the hypercholesterolemic animals, there is at least the suggestion that the effects of magnesium may be mediated via changes in protein and lipoprotein metabolism. Cholesterol and cholic acid feeding resulted in increased alpha and beta globulins which tended to decrease with added dietary magnesium (Table V). Further work upon the influence of magnesium on the synthesis and degradation of the serum proteins and lipoproteins is indicated.

SUMMARY

The variables involved in these studies with weanling rats were the level of dietary magnesium and dietary calcium and the presence or absence of the hypercholesterolemic agents, cholesterol or cholic acid. Measurements were made of growth, serum cholesterol, vascular sudanophilia (heart score), calcification in the kidney tubules (kidney score), kidney weights, and, in some groups, measurement of the serum proteins and lipoproteins.

In hypercholesterolemic animals, additional magnesium invariably decreased the heart score in the animals receiving 600 or 1200 mg per 100 g dietary calcium. This effect was not related to changes in the serum cholesterol level.

Magnesium was ineffective in decreasing the heart score in animals fed the lowest level of calcium, 200 mg per 100 g. Thus, it is concluded that some minimal level of calcium (above 200 mg per 100 g) must be fed for magnesium to be effective in diminishing vascular sudanophilia.

Renal calcification was abolished by raising the level of dietary magnesium and may be independent of the presence or absence of hypercholesterolemic agents.

In the animals fed the cholesterol–cholic acid-free diets, additional magnesium resulted in lower levels of both the alpha and beta lipoproteins. While this effect could not be shown in the hypercholesterolemic animals, it is possible that the effect on vascular sudanophilia may be mediated through the serum lipoproteins.

Serum albumin was consistently decreased

and the alpha and beta globulins increased in animals fed cholesterol and cholic acid. Additional magnesium tended to decrease the latter.

The effects of dietary calcium are less clear. In confirmation of data in the literature and our previous studies, high levels of dietary calcium were usually detrimental to growth on low-magnesium diets and in two experiments intensified the kidney lesions. No specific effect of calcium upon the susceptibility of the vascular system to the deposition of lipid is apparent from these studies. However, unexpectedly high levels of serum cholesterol were observed in animals fed low-calcium diets containing no hypercholesterolemic agents.

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Serum Magnesium, Cholesterol, and Lipoproteins in Patients with Atherosclerosis and Alcoholism

Some Preliminary Observations

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With the Technical Assistance of Doris N. Block and Patricia Connors

ALTHOUGH the incidence of atherosclerosis in chronic alcoholic subjects has been thought to be low,[1,2] this clinical dictum has not been adequately supported. Older studies often failed to distinguish between medial sclerosis and intimal atheromatosis[1] and failed to present experimental data.[2] Recent autopsy observations have been inconclusive and are retrospective in orientation.[3,4] Experimental studies on different animals under varying conditions have produced conflicting results. The ingestion of alcohol afforded some protection against the development of atherosclerosis in rabbits,[5] but was without apparent effect in fowls[6] and intensified the lesions in rats.[7] There is no convincing evidence at this time to indicate that any relationship between alcoholism and the severity of atherosclerosis exists in humans.

The results of measurements of serum cholesterol, lipoproteins, and magnesium in a group of acute and chronic alcoholic patients are presented in this study. The serum cholesterol and beta lipoprotein concentrations. have been shown to be higher, on the average, in patients with coronary heart disease.[8,9] Perhaps, because of the large variations which occur between patients and in the same patient from time to time, the predictive value of these determinations is of a low order in a population such as the United States. However, in the presence of large and consistent differences in the serum cholesterol level between groups, such as Guatemalans[10] or Bantus[11] compared to people in the United States, differences in the rate of development of atherosclerosis have been demonstrated. The blood levels of magnesium and cholesterol may be inversely related. Bersohn and Oelofse[11] reported that the South African Bantu, among whom myocardial infarctions are rare, have significantly higher serum magnesium and lower cholesterol levels than European whites. The possible significance of magnesium in atherosclerosis has been indicated by animal experiments. In growing rats the severity of atherosclerosis produced by cholesterol and cholic acid feeding was greater in the presence of magnesium deficiency.[12] Furthermore, serum magnesium levels have been reported to be low in alcoholics with delirium tremens.[13] These blood constituents were utilized as indices of possible atherosclerosis because of the impos-

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sibility of diagnosing this disease in the absence of its overt manifestations.

MATERIAL AND METHODS

All patients were seen at least once during their hospitalization by one of us (O. M. J.). Patients were classified as acute alcoholics in the presence of signs of delirium tremens. Twenty-one of these patients were tremulous at their worst, 14 developed hallucinations, and one patient was comatose. The diagnosis of chronic alcoholism was based upon the standard history obtained from patients and/or relatives. Other diseases, usually precipitating hospitalization, were often present, including pneumonia in 13, convulsive disorders in 7, various traumatic conditions in 4, and acute pancreatitis in 2. Liver disease was known to be present in less than a third of these patients, but the entire "liver battery" was not obtained in all patients. In 8 of these alcoholics hepatic coma supervened. The diagnosis was based on the characteristic "flapping tremor."[14]

The diagnosis of atherosclerosis was based solely upon either myocardial alterations, confirmed by electrocardiograms, or upon a cerebral thrombosis in the absence of other possible causes for the neurologic signs. Most patients with cerebral thromboses were seen by the neurologic service in routine consultation and the diagnosis confirmed. It is clearly understood that significant atherosclerosis may be present in the absence of all overt signs by which a clinical diagnosis may be made.

The 12 "controls" were healthy-appearing laboratory personnel in the third and fourth decades. Their nutritional status was considered good, and their alcohol intakes were no greater than occasional "social drinks." This group was accepted despite the demonstration at autopsy of significant amounts of atherosclerosis in individuals of these age groups.[15]

The nonalcoholic patients with liver disease had obvious hepatic disorders with evidence of parenchymal involvement. All patients denied alcoholism and their liver involvement was considered generally as not caused by alcohol.

Serum magnesium was determined by EDTA titration according to the method of Hildebrand and Reilley,[16] cholesterol by the method of Carpenter,[17] and protein fractions and lipoproteins by paper electrophoresis.[18] The latter are reported as areas measured by densitometry. Total proteins were measured by the micro-Kjeldahl method.

RESULTS

Table I shows the average serum magnesium, cholesterol, lipoproteins and total protein levels in the control subjects, patients with atherosclerosis, the alcoholics, and the nonalcoholics with liver diseases. The average serum magnesium ranged from 1.69 mg per 100 ml in the patients with atherosclerosis to 1.91 in the controls. The lowest cholesterol levels (174 mg per 100 ml) were seen in the alcoholics and the highest (204 mg per 100 ml) in the controls and the patients with atherosclerosis. None of these differences reach statistical significance. The only statistically significant differences found for alpha lipoproteins ($p<0.05$) are those between the controls and the atherosclerotic patients. The beta lipoprotein values of the atherosclerotics are significantly different from each of the other

TABLE I

Average Serum Levels of Magnesium, Cholesterol, Lipoproteins, and Total Proteins and Its Fractions

	No. patients	Magnesium (mg/100 ml)	Cholesterol (mg/100 ml)	α-Lipoproteins (sq cm)	β Lipoproteins (sq cm)	Total proteins (g/100 ml)	Albumin	Globulin			
								α_1	α_2	β (g/100 ml)	γ
Controls	12	1.91	204	2.2	8.5	7.21	4.50	0.29	0.47	0.81	1.14
Atherosclerotics	23	1.69	204	5.3	11.5	6.81	3.22	0.41	0.74	1.04	1.40
Alcoholics	66	1.85	174	3.9	8.2	6.57	2.89	0.47	0.70	1.08	1.43
Nonalcoholics with liver disease	9	1.76	179	2.6	9.6	6.50	2.68	0.41	0.53	0.80	2.08

TABLE II

Magnesium, Cholesterol, and Lipoprotein Findings in Alcoholic Patients with Atherosclerosis

Patient No.	Age	Sex	Alcoholic intake	Atherosclerotic manifestation	Serum magnesium (mg/100 ml)	Cholesterol (mg/100 ml)	α Lipoproteins (sq cm)	β Lipoproteins (sq cm)
1	67	M	1 pt per day	Remote myocardial infarction	1.98	123	7.0	12.5
3	74	M	"Heavy" until 4 years ago; now "less"	Coronary insufficiency	2.40	192	5.0	14.3
24	71	M	"Heavy"	ASHD* with congestive failure	0.86	134	3.8	8.0
42	85	M	"Moderately heavy	Cerebral thrombosis and ASHD	1.25	194	10.5	9.1
69	63	M	"Moderately heavy"	Cerebral thrombosis	2.16	86	3.7	5.3
173	71	M	"Heavy"	Cerebral thrombosis	0.78	182	5.6	13.4
				AVERAGES	1.57	152	5.9	10.4

* ASHD = Arteriosclerotic heart disease.

TABLE III

Clinical and Laboratory Data Obtained from Alcoholic Patients with Liver Coma

Patient No.	Age	Sex	Date	Serum magnesium (mg/100 ml)	Cholesterol (mg/100 ml)	α Lipoproteins (sq cm)	β Lipoproteins (sq cm)	Clinical status
4	48	M	1/15	1.80	59	1.8	4.7	Tremulous
			1/24	3.27	52	1.8	4.9	Liver flap; lethargic; died in 3 days
6	44	F	1/14	1.74	162	3.5	8.1	Conscious and clear
			2/10	2.32	202	3.1	5.4	Clear; liver flap had been present the day before
			2/21	2.67	126	3.1	7.1	Liver flap; died in 9 days
13	48	M	1/21	2.11	146	5.1	8.9	Stuporous, with signs of severe liver impairment; died next day
105	39	M	3/27	<0.30	345	0.8	12.1	Clear mentally
			3/31	2.40	290	1.0	10.2	Delirium tremens with hallucinations
			4/2	2.64	354	1.1	5.6	Mentally dulled, with liver flap
			4/10	2.60	280	2.6	23.1	Liver coma has cleared
109	38	M	3/31	2.70	152	3.9	8.7	Stuporous
			4/2	1.50	144	4.0	5.5	Confused
			4/8	1.20	138	2.0	7.4	Clearing
129	44	F	4/15	1.04	100	3.2	9.6	Mentally dulled; liver flap not elicited
			4/16	1.30	88	3.6	10.3	Stuporous
			4/18	2.97	104	0.9	5.4	Comatose; oliguria; died next day
138	43	M	4/21	2.37	114	1.5	8.8	Mentally clear
			4/24	3.57	125	2.6	11.5	Disoriented; liver flap
141	34	M	4/22	2.08	283	1.9	3.3	Mentally clear
			4/25	1.91	204	2.0	10.1	Confused and hallucinating with tremor but no flap
			5/5	3.81	132	2.2	9.5	Jaundice increasing; liver flap
NON-COMA AVERAGE				1.41	191	2.2	8.2	
COMA AVERAGE				2.97	149	2.6	7.7	

groups ($p<0.05$). The total serum proteins and their fractions are also tabulated. These are generally comparable except for the gamma globulins, which are elevated in all groups when compared to the controls.

The findings in the six patients in whom alcoholism and atherosclerosis coexisted are summarized in Table II. These patients had been included in Table I with the patients with atherosclerosis, not with the alcoholics. All

TABLE IV

The Effect of Delirium Tremens on Serum Magnesium, Cholesterol, and Lipoproteins

Patient No.	Age	Sex	Date	Clinical status	Magnesium (mg/100 ml)	Cholesterol (mg/100 ml)	α Lipoproteins (sq cm)	β Lipoproteins (sq cm)	Other diseases
2	45	M	1/13	Hallucinating	2.88	142	4.6	8.4	—
			1/20	Clear	2.70	138	2.3	4.4	—
8	35	M	1/16	Tremulous	2.64	198	5.7	6.1	—
			1/17	Clear	1.86	226	5.3	8.9	—
11	48	F	1/20	Tremulous	0.78	230	5.6	4.5	—
			1/23	Comatose	1.77	202	7.6	8.6	—
			1/24	Comatose	1.50	192	7.5	12.2	—
			2/3	Hallucinating	1.70	183	5.1	10.8	—
			2/14	Clear	2.37	216	4.0	12.9	—
12	49	F	1/21	Hallucinating	2.16	138	5.5	6.7	—
			1/23	Clear	1.65	164	1.8	5.4	—
17	48	M	1/27	Tremulous	2.55	178	4.5	8.0	—
			1/29	Tremulous	1.83	146	5.5	11.7	—
			2/3	Tremulous	1.81	152	3.1	7.1	Duodenal ulcer
19	30	M	1/27	Hallucinating	3.30	210	2.5	7.6	Fracture; pneumonia
			1/31	Tremulous	0.96	102	2.9	5.9	Died
38	50	M	2/5	Hallucinating	1.18	224	3.4	3.7	—
			2/7	Clear	2.04	234	6.9	10.5	—
36	68	M	2/5	Tremulous	2.32	226	3.1	5.9	—
			2/6	Tremulous	2.18	218	4.8	6.6	—
40	57	M	2/7	Tremulous	1.44	243	6.4	7.1	—
			2/10	Tremulous	1.82	230	3.7	8.2	—
46	53	M	2/11	Hallucinating	2.16	224	5.7	10.2	—
			2/13	Clear	2.46	206	9.1	11.1	—
50	50	F	2/13	Tremulous	1.62	152	1.7	6.7	Pneumonia
			2/19	Tremulous	2.38	156	5.9	12.8	—
61	45	M	2/25	Tremulous	2.78	168	3.5	3.6	—
			2/27	Tremulous	2.01	160	5.4	8.7	—
			3/3	Clear	1.77	146	4.3	10.2	—
77	45	M	3/10	Tremulous	2.39	156	5.8	6.5	—
			3/11	Clear	2.05	160	5 5	8.5	—
88	48	F	3/17	Tremulous	0.97	228	8.8	7.3	—
			3/18	Clear	<0.3	248	10.4	11.1	—
			3/20	Hallucinating	0.58	234	14.0	15.1	—
			3/24	Clear	0.58	212	5.8	8.8	—
105	38	M	3/27	Clear	<0.3	345	.8	12.1	—
			3/31	Hallucinating	2.40	290	1.0	10.2	—
			4/2	Liver coma	2.64	354	1.1	5.6	—
			4/10	Clear	2.60	280	2.6	23.1	—
108	59	M	3/28	Tremulous	1.80	162	3.8	9.9	—
			3/31	Clear	2.10	173	4.0	7.2	—
120	49	M	4/10	Tremulous	1.16	172	4.6	5.6	—
			4/11	Clear	1.70	197	6.2	9.0	—
121	53	M	4/10	Hallucinating	1.74	148	1.9	6.4	—
			4/11	Confused	1.43	150	6.5	12.9	Pneumonia
			4/14	Tremulous	1.37	122	2.0	8.0	—
132	38	M	4/15	Tremulous	1.83	268	7.2	16.9	—
			4/16	Clear	1.78	260	5.8	18.9	—
141	34	M	4/22	Clear	2.08	288	1.9	3.3	—
			4/25	Hallucinating	1.91	204	2.0	10.1	—
			5/5	Clear; liver flap	3.81	132	2.2	9.5	—
143	50	F	4/23	Tremulous	qns	146	8.0	9.3	—
			4/24	Hallucinating	1.19	255	6.3	6.1	—

TABLE IV (*Continued*)

Patient No.	Age	Sex	Date	Clinical status	Magnesium (mg/100 ml)	Cholesterol (mg/100 ml)	α Lipoproteins (sq cm)	β Lipoproteins (sq cm)	Other diseases
143, *cont.*			4/25	Clear	1.09	157	6.2	6.3	? Drinking ward alcohol
			4/28	Clear	2.18	186	3.7	6.5	—
155	43	M	5/8	Tremulous	1.91	182	3.9	7.7	—
			5/9	Hallucinating	1.63	178	4.4	10.2	—
			5/12	Clear	1.63	182	3.9	11.9	—
156	55	M	5/8	Clear	2.72	156	3.0	7.8	Subtotal gastrectomy, remote
			5/9	Hallucinating	1.63	162	2.9	8.8	—
			5/12	Hallucinating	3.00	184	1.2	7.9	—
			5/20	Clear	1.31	206	2.0	11.9	? Marginal ulcer
163	45	M	5/19	Hallucinating	1.36	154	5.7	5.8	—
			5/20	Tremulous	1.05	160	3.7	5.6	—
				Admission Average	1.90	197	4.5	7.4	—
				Final Average	1.92	186	4.2	9.7	—

admitted to heavy alcoholic intakes, but one patient claimed he had lessened his intake during the preceding four years. Data on dietary histories are scanty. Several patients admitted inadequate food intake but only one patient was considered by clinical examination to be "not well nourished." In this group the serum magnesium levels averaged 1.59 mg per 100 ml and the cholesterol 152 mg per 100 ml, both lower than the corresponding values in the alcoholics and the atherosclerosis group as a whole. The alpha lipoprotein was 5.9 sq cm, and the beta 10.4 sq cm, approximately equal to that of the entire atherosclerosis group and higher than that in the alcoholics.

In Table III are the results obtained in eight alcoholic patients in whom severe liver disease was present, leading to the development of hepatic coma. In one patient, OJ-109, coma was present initially and there was subsequent gradual improvement. The noncoma average for magnesium was 1.41 mg, cholesterol 191 mg, both per 100 ml, alpha lipoproteins 2.2 sq cm; and beta lipoproteins 8.2 sq cm. In comparison to the values observed in the alcoholics, the magnesium and alpha lipoproteins are lower, and the cholesterol and beta lipoproteins are higher in the precoma patients. With the development of hepatic coma there was a marked increase in the serum magnesium (or, conversely, a pronounced decrease in serum magnesium in the patient with clearing of the coma). The cholesterol levels fluctuated in the individual patients but evidenced a tendency to decrease and fell to an average of 149 mg per 100 ml during coma. The alpha lipoproteins were essentially unchanged. The beta lipoproteins varied with gross irregularity, and the average during coma was 7.7 sq cm, which was somewhat less than the average for all alcoholics.

In Table IV the results in 24 alcoholics with delirium tremens are compared at admission and following varying periods of hospitalization. The initial levels of serum magnesium, cholesterol, and lipoproteins generally corresponded to the time of the most intense signs of alcoholic delirium. Of these patients, 1 was comatose, 13 had hallucinations, and 10 were tremulous. At the time of the final determination all patients were improving and most were clear mentally. The final averages are similar to the initial levels, except for some increase in the beta lipoproteins from 7.4 to 9.7 sq cm. These similarities occurred in the face of extensive individual variability. With clinical improvement in the intensity of the delirium the serum magnesium fell in 11 patients, increased in 8. In 6 patients with clinical deterioration during hospitalization, the magnesium increased in 3 and declined in 3. Both the beta lipoprotein and serum cholesterol also

varied—the lipoproteins increased in 17 and fell in 7 and the cholesterol increased in 10 and fell in 13 patients. However, there was no consistent pattern in these alterations and no correlation was apparent among the changes or between them and such clinical phenomena as improvement or worsening, return of appetite and increased food intake, or the preceding nutritional history or status of the patients.

DISCUSSION

Although these preliminary results do not answer the old question regarding the frequency of atherosclerosis in alcoholics, the data contain no suggestion that the alcoholic is spared the rigors of atherosclerosis. There is no significant difference between the cholesterol and beta lipoprotein levels in the few controls reported and the alcoholics, nor do the serum cholesterol values appear grossly different from "normal" values appearing in the literature.[8,9] This is consistent with an equal predisposition toward atherosclerosis. Clinical evidence of atherosclerosis did occur in 6 of our alcoholics. None of these exhibited abnormally high serum cholesterol values.

These 6 alcoholics with atherosclerosis are considerably older than the average of the entire group of alcoholics. A time factor has been operative and their survival beyond 63 years allowed them to arrive in the decades in which atherosclerosis becomes frequent. The absence of advanced complications of alcoholism in these patients is significant, at least in permitting this survival to occur. However, the data do not indicate that one such complication, liver disease, has any effect on the occurrence of atheromatous disease, other than the possible shortening of life. The serum beta lipoproteins and cholesterol levels in the alcoholics with liver coma are essentially the same as those in the alcoholic group as a whole. Similarly, in the nonalcoholics with liver disease the cholesterol and beta lipoprotein values are not grossly different from the controls or alcoholics.

The relationship between serum cholesterol or beta lipoproteins to coronary atherosclerosis has been studied more extensively than have these two variables and cerebral atherosclerosis. Epidemiologic studies have indicated that in certain areas of the world, particularly in Japan,[19] there exists a marked difference in frequency between coronary and cerebral atherosclerosis, the latter being more prevalent. The validity of causes of death obtained from death certificates is open to question.

Morphologically, however, the atherosclerotic lesions of both the coronary and cerebral arteries are similar. For this reason our patients with cerebral thrombosis were considered part of this atherosclerotic group. In any event, the exclusion of this small group of patients would not alter the results of this study.

The variations in serum cholesterol and magnesium levels in the same patient were often larger than might be expected. When a serum magnesium level appeared unusually low, the serum was reanalyzed for magnesium. However, none of the methods usually used for the magnesium determinations have been extremely reliable in our laboratory. Repeated determinations upon a series of serums resulted in a standard deviation of 0.2 mg per 100 ml. If the outside limits are considered to include three standard deviations then a serum containing 1.0 mg per 100 ml might occasionally yield a value as low as 0.4 mg per 100 ml. Hence, individual determinations cannot be considered too seriously. On the other hand, it is clear that the method is adequate to demonstrate average differences in groups of patients. This is demonstrated in the present data by the rise in serum magnesium in patients developing hepatic coma. The concomitant changes in serum cholesterol and lipoproteins were not consistent.

In our patients with delirium tremens serum magnesium was frequently within normal limits and, despite considerable individual variability, there was no significant change in the group average with hospitalization and clinical improvement. We have been unable to relate serum magnesium to the development of delirium tremens.

The elevation of gamma globulins in the atherosclerotic patients was unexpected. It cannot be explained solely on the basis of liver disease, since it occurred also in its absence.

CONCLUSIONS

The results of this study do not support the concept that overt atherosclerosis is less frequent in the chronic alcoholic. No significant differences in serum cholesterol and lipoprotein fractions were found.

We have not been able to demonstrate a correlation between serum magnesium and serum cholesterol levels. Beta lipoproteins were elevated in the atherosclerotic group.

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Effect of Mixed Fat Formula Feeding on Serum Cholesterol Level in Man

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IN EXPERIMENTAL STUDIES with cholesterol–cholic acid-fed rats, Hegsted *et al.*[1] found an apparent synergism between the completely saturated fatty acids and linoleic acid in the lowering of the levels of serum cholesterol. The product of the saturated fatty acid content times the linoleic acid content of the dietary fat had a high inverse correlation with the serum cholesterol level, when a number of oils and mixtures of oils were tested. This finding is apparently incompatible with the results obtained by Ahrens and associates,[2] Keys *et al.*,[3] and others who have studied the effects of dietary fats upon the level of serum cholesterol in man. In general, increasing the saturated fatty acids in the diet apparently causes an increase in the serum cholesterol level, although all studies have not uniformly shown this effect.

In the studies upon rats an equal mixture of coconut oil and safflower oil gave the highest product of the mixtures studied and provides about maximum amounts of linoleic acid and saturated fatty acids in readily obtainable forms. The admixture results, of course, in an oil with approximately half the iodine number and about half the linoleic acid content of safflower oil, since coconut oil is nearly devoid of unsaturated acids.

The present paper presents the results obtained upon a series of 10 patients in which a formula diet was used and in which the two fats, safflower oil and an equal mixture of safflower and coconut oils, were compared.

The difficulties encountered in interpreting changes in serum cholesterol are well known to those interested in this field of investigation. We believe the experimental design used is efficient and useful for the comparison of two dietary effects, since it avoids the subjective determination of "plateau" levels of serum cholesterol and randomizes the effects of variables which may depend upon the time determinations are done.

MATERIAL

The formula diet which was used in these experiments was similar to that of Ahrens *et al.*[2] Safflower oil or an equal mixture of safflower and coconut oils constituted 42 per cent of the total daily calories, while carbohydrate in the form of glucose, and protein derived from milk proteins, contributed 43 per cent and 15 per cent of the calories, respectively. Sodium chloride (2 g) and two multivitamin capsules were given as daily supplements.

The pertinent clinical data on the patients who participated in this study are presented in Table I. All of the subjects were hypercholesteremic; two had xanthomata and 8 had compensated coronary heart disease and healed myocardial infarction. All patients were ambulatory. During the experimental period, body weights were kept constant by adjustment of the formula intake. Preced-

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TABLE I
Clinical Data on Male Patients with Hypercholesterolemia

Case no.	and initials	Age	Occupation	Height (inches)	Present weight (lb)	Clinical diagnosis	Maximum weight (lb)
1	W. M.	35	Draftsman	70	153	C.H.D., M.I.	194
2	J. P.	35	Policeman	67	145	C.H.D., M.I.	186
3	J. G.	50	Gardener	65	146	C.H.D., M.I.	190
4	S. S.	40	Police captain	69	180	T.X.	220
5	J. B.	32	Postal clerk	73	197	C.H.D., M.I.	235
6	F. S.	45	Lawyer	70	150	C.H.D., M.I.	160
7	J. F.	42	Mechanic	68	163	C.H.D., M.I.	190
8	J. D.	41	Photographer	63	180	M.X.	201
9	T. F.	50	Clerk	66	163	C.H.D., M.I.	194
10	J. D.	42	Heavy-machinery operator	64	160	C.H.D., M.I.	169

C.H.D. = coronary heart disease; M.I. = myocardial infarction, stable; T.X. = tendon xanthomata; M.X. = mixed xanthomata.

ing the present study by two months to eight years, a fairly constant body weight had been maintained in all of the patients with coronary heart disease since their convalescence from acute myocardial infarction.

Nine of the patients had been obese prior to the development of their disorders We have obtained careful dietary histories from all of these patients over the years preceding their difficulty. A summary of these dietary intakes is presented in Table II. As revealed from the statistical analysis, a considerable amount of variation occurred in the constituents of these high calorie diets. Nonetheless, the variations were found at higher levels of intake. We emphasize that these figures are approximations derived from dietary histories, yet they undoubtedly played a role in the attainment of each individual's "maximum" obese weight.

METHODS

The studies were made on patients in the metabolic ward. Prior to the start of the formula feeding, two blood samples were drawn approximately 24 hours apart for cholesterol analysis, which was done by the method of Abell *et al.*[4] in duplicate. Alternate patients were assigned to either group A or group B, and the experimental study began

TABLE II
Summary of Daily Dietary Intake of the 10 Reported Patients Over the Years Prior to Development of Clinical Disorder

	Calories	Carbohydrate (g)	Protein (g)	Total fat (g)	Animal fat (g)	Vegetable fat (g)	Alcohol (g)
Mean	5,673	545	173	252	197	55	75
S.D.*	±1,919	±201	±64	±68	±83	±27	±75
S.E.†	±606	±63	±20	±21	±26	±8	±24

* Standard deviation. † Standard error.

after the second control sample was taken. Groups A and B were similar except for the order of the two oils tested. Those in group A received the safflower oil formula followed by a similar period of the mixture of safflower and coconut oils, while group B received the mixture first followed by the safflower oil alone. In the beginning it was planned that the feeding periods on each oil would be of three weeks' duration, but in approximately half of the patients the time had to be decreased to two weeks, since a maximum of one month was as much time as could be obtained from many of the men. On the last two days of the feeding period, blood samples were obtained and analyzed as before. The formula was changed and the procedure repeated.

RESULTS

The results of this study are shown in Table III, where the mean cholesterol values for each man at the beginning and at the end of each period are presented. It can be seen that

TABLE III
Mean Cholesterol Values

Group and subject		No. weeks	Mean serum cholesterol (mg/100 ml)		
			Control	Safflower	Mixture
A	1	3	364	215	214
	3	3	358	279	272
	5	2	353	250	281
	7	2	336	232	240
	9	3	315	276	198
Mean			345	250	241
			Control	Mixture	Safflower
	2	3	416	274	251
	4	2	348	245	217
	6	2	331	265	285
	8	3	489	361	351
	10	2	310	289	257
Mean			379	272	287
			Control	Safflower	Mixture
Grand mean			362	268	256

either safflower oil or the mixture of safflower and coconut oils caused a marked decrease in the serum cholesterol levels and approximately to the same extent. Indeed, the mixture of oils caused a slightly greater decrease in the cholesterol level than did safflower oil.

A variance analysis of the results is shown in Table IV. The so-called "treatment effect" including the control and two feeding periods is highly significant. Nearly all of this variance is accounted for by the difference between the control period and the two oil periods. The difference between the two oil periods is not significant. The data also show, as would be expected, that there are significant differences in the serum cholesterol levels of the individuals tested. The variation between pairs of determinations, i.e., samples of blood taken a day apart at the beginning and at the end of each feeding period, gives a standard deviation of about 10 mg/100 ml. This would be representative of the reproducibility of the serum cholesterol value on separate blood samples.

TABLE IV
Analysis of Variance

Source of variation	Degrees of freedom	Sum of squares	Mean square	F
Treatment	2	133,536	66,768	44.8*
Control vs. oils	1	132,202	132,202	88.7*
Between oils	1	1,334	1,334	—
Interaction	18	26,817	1,489.8	—
Between individuals	9	88,465	9,829.4	88.05*
Within pairs of determinations	30	3,349	111.63	—

* Significant; $p < 0.01$.

DISCUSSION

The results of this study are in essential agreement with the previous results obtained with rats.[1] With the number of patients tested one could not expect to show significant differences in the potency of oils when the results are as closely similar as those obtained. Nevertheless, it is of some interest that the mixture of oils, in both series,

gave slightly lower serum cholesterol values than safflower oil alone.

The results appear at the moment to be incompatible with the generalizations proposed by Ahrens *et al.*[2], Keys *et al.*,[3] or other workers in the field. Whereas Ahrens and co-workers[2] found the "serum cholesterol lowering effect" to be closely related to the iodine value of the dietary fat, the mixture of coconut oil with an iodine value of 10 to safflower with an iodine value of 145 yields a mixture with an iodine number of 78. Similarly, applying the formulation of Keys and associates,[3] in which saturated fatty acids counteract the action of polyunsaturated fatty acids, does not account for the results obtained. The mixture provides 51 g of saturated acids and approximately 42 g of polyunsaturated acids per 100 g compared to 12 and 81 respectively in safflower oil and, according to Keys' formulation, should produce considerably higher serum cholesterol levels. Since this work was done with hypercholesterolemic patients the actual coefficients in Keys' formula may not be applicable. Kinsell *et al.*[5] concluded that the major cholesterol lowering ingredient in various vegetable fats was linoleic acid. The results are not compatible with the belief that the linoleic acid content of an oil is proportional to its lowering effects upon serum cholesterol.

This does not appear to be the appropriate place to discuss the many and diverse findings so far reported in the literature. Still, it is worthwhile to note that, contrary to the usually accepted opinion, various workers have found that the ingestion of hydrogenated fats does not invariably result in a rise in the serum cholesterol. Bronte-Stewart and associates[6] also have reported one case in which the addition of hydrogenated ground-nut oil to a highly unsaturated fraction of sunflower seed oil did not apparently effect a rise in the serum cholesterol level. Hydrogenated coconut and whale oils did not affect serum cholesterol when fed individually as the sole source of fat,[7] and hydrogenated corn oil added to butterfat produced a hypocholesterolemic response.[8]

It is apparently true at this time that none of the generalizations so far proposed can account for the diverse results obtained in different laboratories. Nor does it seem likely that this diversity can be explained as being due to "errors." It appears more likely that the differences in conditions in the various laboratories—basal diets, patients used, etc.—may account for some of the discrepancies. Since the conditions of our studies are similar to those employed by Ahrens, it may be worthwhile to inquire whether mixtures of fats behave in a manner similar to natural fats of the same general fatty acid composition. However, we may note that the mixture of safflower and coconut oils falls into an area upon the triangular plot of Ahrens which is blank in his paper. If these data are comparable to those of Ahrens, i.e., safflower oil is essentially the same as corn oil and the mixture is similar to safflower oil, then the curved surface should drop rapidly along the saturated-linoleic coordinate, reaching a minimum by at least the 50-50 point.

In spite of much work in recent years, the adequate determination of serum cholesterol levels remains a problem. In few laboratories are there sufficient controls in operation to determine the deviations from the true levels occasioned from day to day, week to week, between technicians using the same method, etc. Experimental designs should be utilized in which such variation cannot possibly influence the results. The subjective evaluation of "constant" or plateau levels should also be eliminated if possible.

The mechanism of the apparent synergism between the saturated and the essential fatty acids in lowering serum cholesterol remains unknown. Preliminary experiments indicate a difference in the degree of cholesterol response to the mixture of oils related to the initial body weight of the experimental subject. It is not known whether the initial depot fatty acid composition determines the body's response toward a critical mixture of fatty acids. It is possible that the initial cholesterol lowering usually obtained by consuming a generous quantity of corn or safflower oil may be in part due to a synergism

between it and the saturated fatty acids of body fat.

We believe that the results obtained here are important insofar as they suggest caution against the belief that the "saturated" fats are "bad" and the essential fatty acids are "good." In addition, they emphasize the need for investigation of the potency of mixtures of oils in man, which may lead to more acceptable dietary regimens which are not a "compromise" but actually desirable.

SUMMARY

Formula-feeding experiments were conducted in 10 patients with hypercholesterolemia, in which the two fats, safflower oil and an equal mixture of safflower and coconut oils, were compared with regard to their effect on serum cholesterol level.

Either the safflower oil or the mixture of safflower and coconut oils caused a marked decrease in serum cholesterol. The mixture effect was obtained regardless of whether it was fed before or after the safflower oil. The results are comparable to those previously obtained with these two oils in rats.

The results are incompatible with the proposed hypotheses that the serum cholesterol-lowering effect of a dietary fat is proportional to the iodine value or the linoleic acid content of the fat. In addition, the results do not support the view that the saturated fats counteract the effect of polyunsaturated fats.

The experimental design used is efficient and useful for the comparison of two dietary effects on serum cholesterol level in man. It eliminates the subjective evaluation of constant or plateau levels of serum cholesterol and randomizes the effects of variables inherent in time and methodology of determination of the lipid.

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The Effects of Various Vegetable Oils on the Serum Lipids of Adult American Males

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THERE IS evidence[1-3] that an association exists between increased serum total cholesterol and atherosclerosis. Some investigators[4,5] maintain that serum total cholesterol increases as the relative amount of dietary fat increases. However, certain dietary fats have been shown to depress serum total cholesterol in human subjects. Groen[6] reported the lowering of serum cholesterol levels in man by the ingestion of a vegetarian diet, even if the fat content of the diet were high. Other researchers[7-9] have observed that the ingestion of formula diets containing relatively large amounts of unsaturated vegetable fat resulted in a fall in serum cholesterol.

Bronte-Stewart *et al.*[10] compared the effects of various animal and vegetable fats on serum cholesterol levels in man. Their findings supported their hypothesis that serum lipids are controlled by the alteration of saturated and unsaturated fats in the diet rather than by the source of the fat per se. The results of these and other experiments have led to speculation[11] that lipidemia is associated with a relative deficiency of essential fatty acids. Keys[12] demonstrated reductions in serum cholesterol levels by decreasing the percentage of total calories supplied by saturated fatty acids and increasing the percentage of calories from polyethenoid fatty acids independent of the percentage of calories supplied by monoethenoid fatty acids and total fat.

The effects on serum total cholesterol of the inclusion of two kinds of vegetable oil preparations*,† in the diets of adult males living at home and choosing their own diets are reported here. In the first study, conducted in the fall of 1955, 20 men included eight ounces of an emulsified peanut oil preparation* in their diet for a four-week period. In the fall of 1957, 10 of

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* Lipomul-Oral, Upjohn Co., Kalamazoo, Mich. This was a homogenized emulsion of vegetable oils containing 36 per cent peanut oil, 4 per cent coconut oil, and 10 per cent anhydrous dextrose in 3 per cent emulsified vehicle, lecithin; butylated hydroxyanisole; alkyl aryl polyether alcohol; water; and a trace of hydrochloric acid and preserved with 0.1 per cent sodium benzoate. Since this study, Lipomul-Oral has been revised and now contains 66 per cent corn oil in place of peanut and coconut oil, along with emulsifying, flavoring, and sweetening agents but no sugar.

† Arcofac, Armour & Co., Chicago: This is a safflower oil emulsion containing in each tablespoon (15 cc) 6.8 g linoleic acid, 0.6 mg pyridoxine hydrochloride, 11.5 mg mixed tocopherols, and sodium benzoate as a preservative. Linodoxine, Pfizer & Co., Brooklyn: This is a safflower oil emulsion containing in each tablespoon (15 cc) 4.5 g linoleic acid, 5 mg pyridoxine hydrochloride, 20 mg mixed tocopherols, and glycerine as a stabilizing agent.

these men took 1½ ounces of commercial safflower oil preparations (rich sources of linoleic acid) (see footnote† on p. 35) for four weeks.

METHOD

The subjects were members of the faculty of the Massachusetts Institute of Technology. Approximately 50 men who were found to have serum cholesterol and/or lipoprotein levels above the mean for their age[13] during their annual physical examinations in 1952–1954 were invited in 1955 to participate in the first study reported here. Of the 25 men who volunteered, 20 completed the four-week dietary regimen. One subject was unavailable at the time blood samples were drawn; four other subjects had incomplete diet records or were unable or unwilling to continue. These five men were excluded from the tabulations. The ages of subjects ranged from 37 to 65 years, with a mean age of 47.8 years. Half the subjects were under and half were over the mean age.

A Burke-type research dietary interview[14] was taken. In addition, each man recorded his food intake in detail during the control period and for the first week of the experiment.

The men were given a supply of the emulsified peanut oil and instructed to take eight ounces of the oil daily for four weeks. It was suggested that the emulsion be taken in three doses of approximately 80 ml before each meal. This amount of the emulsion supplies approximately 960 calories, 96 g fat, 24 g carbohydrate, and 23 g linoleic acid.

The subjects were asked to keep their body weight constant. This necessitated the omission of some foods, usually visible fats (butter, cream, whole milk, margarine, gravies, fried foods, mayonnaise, and fat on meat), from their diets.

Two samples of blood for control measurements were obtained at an interval of one week. The subjects were weighed when the samples were taken. Blood samples were collected and weights were recorded weekly during the experimental period. The relative weights of the subjects were computed, using the tables of desirable weight of the Metropolitan Life Insurance Company.[15] Serum total cholesterol was measured by the method of Abell and her associates.[16] The serum lipoproteins were determined by the method of Gofman *et al.*[17] on the first control sample and after the fourth week of emulsion feeding.

In the fall of 1957, 16 of the men who had participated in the 1955 emulsion study volunteered as subjects for another study, in which each man took three tablespoons of a commercial safflower oil emulsion daily for a four-week period. (Of the original group of 20 men from the previous study, two were out of the country, one faculty member had resigned, and a fourth did not wish to participate.) Because of travel commitments or illness, 6 of the 16 subjects were absent two or more times when blood samples were collected or the control or final samples were collected. These six subjects were therefore excluded from the study.

The men were given randomly a supply of one of two commercial safflower oil preparations and were instructed to take three tablespoons of the oil daily for four weeks. This amount of one preparation supplies approximately 250 calories, 28 g of fat, and 20 linoleic acid. An equal amount of the other preparation supplies approximately 210 calories, 23 g fat, and 13.5 g linoleic acid.

The second study was conducted in the same manner as the 1955 experiment with the following exceptions:

(1) The subjects recorded their food intake in detail during the control week and for the entire four weeks of the experimental period. A research dietary interview was not taken during the 1957 study.

(2) The quantity of fat preparation taken in the second study was much smaller (1½ ounces daily) than in the first study (8 ounces daily).

(3) Serum total cholesterol was measured by the micro-method of Carpenter, Gotsis, and Hegsted[18] of our laboratory. The results obtained by this micro-method are in good agreement with those obtained with the standard colorimetric method of Abell *et al.*[16], which was used in the 1955 study.

RESULTS

1955 Study

A summary of the dietaries of the subjects,

TABLE I

Mean Values and Standard Deviation of Certain Dietary Components of a Selected Group of Men

	1955 Study		1957 Study	
	Past dietary history	1-wk experimental period	1-wk control period	4-wk experimental period
No. men	20	20	10	10
No. calories	2,460 ± 400	2,625 ± 250	2,055 ± 420	2,330 ± 365
Fat				
% of calories	45 ± 5	56 ± 5	39 ± 6	45 ± 4
% animal origin	76 ± 8	33 ± 9	75 ± 10	58 ± 5
Cholesterol (mg)	707 ± 225	497 ± 200	546 ± 46	555 ± 205
SFA*				
% of calories	18.3 ± 2.4	17.4 ± 0.9	15.2 ± 3.1	13.4 ± 2.1
SFA/UFA†	0.75 ± 0.09	0.48 ± 0.08	0.70 ± 0.12	0.48 ± 0.08
MFA‡				
% of calories	19.6 ± 1.3	25.7 ± 2.2	17.0 ± 2.9	16.2 ± 1.9
PFA§				
% of calories	5 ± 1	10 ± 2	5 ± 1	11 ± 2
% of fat	11 ± 3	18 ± 2	12 ± 2	24 ± 4
Protein				
% of calories	15 ± 2	11 ± 2	16 ± 2	14 ± 1
% animal origin	80 ± 5	79 ± 5	74 ± 9	76 ± 2
Alcohol				
% of calories	5 ± 4	2 ± 3	6 ± 7	5 ± 6

* Saturated fatty acids. † Unsaturated fatty acids. ‡ Monoethenoid fatty acids. § Polyethenoid fatty acids.

including the usual intake calculated from the dietary histories and the intake during the first week of the experimental period estimated from records kept by the subjects, is presented in Table I.

Because of the prescribed inclusion of the emulsified vegetable oil and the restriction of much of the visible fat from animal sources from the diet, the source of dietary fat was markedly changed. As shown in Table I, approximately 76 per cent of the fat in the subjects' usual diets was of animal origin. This amount was reduced to 33 per cent in the experimental diets. Likewise, other fat components were changed including the cholesterol content of the diet, which was reduced from an average of 707 mg per day to 497 mg. Since the peanut oil emulsion was a relatively good source of unsaturated fatty acids, the mean ratio of saturated to unsaturated fatty acids was reduced 33 per cent (i.e., from 0.75 to 0.48). Essential fatty acid intake increased about 100 per cent. As fat contributed more of total calories, carbohydrate decreased as a source of calories. No other major changes in diet were observed.

The means and standard deviations for serum cholesterol and for the lipoprotein fractions S_f 12–20 and S_f 20–100 for each bleeding are presented in Table II. The general trend observed in the serum cholesterol readings showed a sharp drop after the first week of fat feeding followed by a slight rise toward pre-experimental levels during the second and third week and then a decrease by the end of the fourth week to the level observed at the end of the first week.

The two control readings for serum cholesterol for each subject were averaged. The mean cholesterol of the control readings for the 20 subjects was 290 mg per 100 ml (S.D. ± 42 mg per 100 ml). The mean cholesterol of the last two samples taken after three and four weeks of fat feeding was 259 mg per 100 ml (S.D. ± 38 mg per 100 ml). This represented a mean decrease of 31 mg per 100 ml. This difference was tested and found significant at $p < 0.01$. An analysis of variance showed that the cholesterol level varied significantly ($p < 0.05$) between the two control and four experimental readings. Of the 20 subjects, 2 had an increase in serum cholesterol, 1 showed no change, and the remaining 17 exhibited a decrease. There was a tendency for an indi-

TABLE II

Mean Values and Standard Deviations of Certain Serum Lipids of a Selected Group of Twenty Males Treated with Eight Ounces Emulsified Peanut Oil Orally for Twenty-eight Days (1955 Study)

Time interval	Total cholesterol (mg/100ml)	Lipoproteins	
		S_f 12–20 (mg/100ml)	S_f 20–100 (mg/100ml)
First control	290 ± 48	57 ± 19	97 ± 63
Second control	289 ± 40	—	—
Mean of two controls	290 ± 42	—	—
One week of treatment	255 ± 41	—	—
Two weeks of treatment	264 ± 44	—	—
Three weeks of treatment	263 ± 40	—	—
Four weeks of treatment	254 ± 45	38 ± 24	80 ± 47
Mean of third and fourth weeks of treatment	259 ±38	—	—

Note: The mean values are followed by standard deviation of the distribution.

vidual's serum cholesterol to vary more during the experimental period than during the control period. A trend for the greatest decreases to occur in subjects with the highest control cholesterol levels was indicated by a negative correlation ($r = -0.44$) between the control cholesterol level and the change in cholesterol expressed in milligrams per 100 ml.

An arbitrary division of the subjects into two groups was made on the basis of the per cent change in cholesterol level between the mean of the two control and the mean of the last two experimental readings. Group A includes eight subjects whose cholesterol levels either decreased less than 10 per cent, showed no change, or increased. This group had a mean decrease in serum cholesterol of 1.8 per cent (±5.7 per cent), or, expressed in mg per 100 ml, a drop of 6.3 mg (±14.5). The remaining twelve men, group B, exhibited a decrease of more than 10 per cent in their serum cholesterol. The mean decrease in group B was 16.0 per cent (±3.7 per cent), representing an average decrease of 48 mg cholesterol (±12.1 mg).

Four of the subjects maintained their weight plus or minus one pound. Twelve subjects had a plus or minus two-pound variation, five lost two pounds, while seven gained two pounds. Four of the subjects gained three or more pounds during the four-week period. The maximum weight gain was four pounds. When nine subjects who had either lost weight or had not gained more than one pound were compared to those who had gained two or more pounds, no differences were observed in serum cholesterol decrease.

Since the detailed dietary records were maintained for only one week of the experimental period, no attempt was made to correlate changes in blood findings with levels of specific nutrients.

While there was a trend for group B to be younger, less obese, and to gain less weight than group A, none of these differences were of statistical significance. Group B was 46.7 years old ± 7.5 years as compared with 49.5 ± 9.5 years for group A; group B had a relative weight of 109 per cent as compared with 115 per cent from group A; and the B group gained 0.4 pound as compared with 1.8 pounds for group A.

The mean decrease of 19 mg per 100 ml which occurred in the S_f 12–20 fraction was significant at $p < 0.01$ when the students' "t" test was applied. The mean decrease (17 mg per 100 ml) in the S_f 20–100 fraction was not significant.

1957 Study

The dietaries calculated from diet records kept for five weeks (one-week control period and four-week experimental period) by the ten subjects are summarized in Table I.

The safflower oil preparations contributed approximately 25 g of vegetable fat daily to the diets of the subjects. During the experimental period the men were estimated to be receiving approximately 45 per cent of their calories from fat, as contrasted to 56 per cent during the 1955 study. Due in part to the fact that safflower oil is a rich source of linoleic acid, polyunsaturated fatty acids contributed approximately one tenth of the total calories in both experiments and accounted for almost one-fourth of the total fat in the diet during the 1957 study. No at-

TABLE III

Mean Values and Standard Deviations of Serum Total Cholesterol of a Selected Group of Ten Males Treated with Safflower Oil Preparations for 28 Days (1957 Study)

Time interval	Total cholesterol (mg/100 ml)
First control	267 ± 39
Second control	261 ± 40
Mean of two controls	264 ± 37
One week of treatment*	263 ± 44
Two weeks of treatment*	257 ± 41
Three weeks of treatment	239 ± 50
Four weeks of treatment	233 ± 39
Mean of third and fourth week of experimental period	236 ± 42

Note: The mean values are followed by standard deviation of the distribution.

* Value for 9 subjects. One subject unable to be present at time blood samples were collected.

tempt was made to correlate individual serum cholesterol changes with levels of specific nutrients.

The data for the serum cholesterol for this second study are given in Table III. The two control readings for serum cholesterol for each subject were averaged. The mean cholesterol of the control readings for the ten subjects was 264 mg per 100 ml (S.D. ± 37 mg per 100 ml). The mean cholesterol of the last two samples (i.e., after three and four weeks of fat feeding) was 236 mg (S.D. ± 42 mg per 100 ml). This represented a mean decrease of 28 mg per 100 ml. This difference was tested and found significant at $p < 0.01$. No significant differences were observed in the response of the group fed the two safflower oil preparations.

The subjects were again divided into two groups, i.e., those who exhibited a decrease in serum cholesterol of less than 10 per cent or an increase, and those who had a decrease of 10 per cent or more. The mean decrease for the first group (four subjects) was 2.6 per cent (±6.0 per cent) or 5 mg cholesterol ± 15 mg, while the second group (six subjects) had a mean decrease of 15.8 per cent (±5.1 per cent), representing a decrease of 43 mg of cholesterol ±15 mg.

The behavior of the serum cholesterol of these ten subjects during the 1955 study was compared with the 1957 results. No significant differences were observed between the control cholesterol readings in 1955 and 1957. In the peanut oil study the maximum response occurred after one week of treatment with levels rising toward control levels over the last three weeks of the study. In the 1957 study, however, essentially no change was observed until after three and four weeks of treatment.

Two subjects gained weight during the experimental period of the 1957 study, one and two pounds respectively. Five subjects either maintained their weight for the five-week period or lost one pound. The three remaining subjects lost four, six, and eight pounds, respectively. Weight loss has been associated with a decrease in serum cholesterol.[19] In this study, however, no association was observed between weight changes and serum cholesterol levels. For example, the average control serum cholesterol value of the individual who lost eight pounds was 313 mg per 100 ml. His average value after three and four weeks of treatment was 301 mg per 100 ml, representing a 3.8 per cent decrease in serum cholesterol. On the other hand, the subject who gained one pound experienced a 25 per cent decrease in serum cholesterol.

DISCUSSION

Both the generous inclusion of peanut oil (approximately one-third of the total calories and two-thirds of the total fat) in the diet of the subjects and the daily use of three tablespoons of commercial safflower oil preparations seemed effective in slightly lowering serum cholesterol. However, only 60 per cent of the subjects (12) in the peanut oil experiment and a like percentage (6 subjects) in the safflower oil studies had an actual decrease of more than 10 per cent in the serum cholesterol, and 40 per cent (8 in 1955 and 4 in the 1957 study) showed either no change or an increase. It must be remembered that spontaneous variations in serum cholesterol and the various lipoprotein fractions are known to occur, and the variability of these measurements in persons with high levels of lipids has been shown to exceed those of individuals with low levels.[20] The extent of the yearly variability of serum cholesterol of the subjects in this study is illustrated in Table IV. Most of these cholesterol values were deter-

TABLE IV

Total Serum Cholesterol (mg/100 ml) of 20 Adult American Men Over a Six-year Period

Subject	1952	1953	1954	1955 Control	1955 Experimental period					1956	1957 Control	1957 Experimental period				
1	—	309	363	409	383	347	380	377	363	405	411	—	—	—	—	—
3	—	—	—	292	295	255	264	286	220	252	Out of country					
4	227	243	224	208	232	216	243	237	247	209	243	269	273	234	202	210
5	281	278	291	303	296	246	246	242	260	—	Left M. I. T.					
6	321	294	366	307	300	280	310	287	300	319	284	268	280	294	Out of town	278
8	254	312	372	362	315	287	305	292	287	338	317	309	292	294	302	300
9	—	246	287	333	320	300	336	305	349	294	302	306	335	321	300	231
11	321	282	370	285	300	227	223	234	218	290	270	240	248	232	210	230
12	303	318	299	313	302	284	245	241	254	286	Out of country					
13	202	243	193	233	240	230	219	235	242	—	206	199	201	226	190	191
14	246	231	—	272	277	232	240	242	219	249	277	268	263	256	252	226
17	212	242	238	244	257	222	227	218	208	225	158	Ill	259	228	218	230
18	304	316	281	301	298	249	251	263	275	297	293	283	292	292	316	296
19	—	310	308	307	325	307	285	278	226	307	270	283	Out of town			
20	240	265	—	290	275	264	261	284	264	247	281	275	270	Tube broke	Out of town	239
21	—	227	265	259	249	225	229	247	239	281	279	228	197	Out of town	236	209
22	184	229	180	218	225	194	229	198	209	244	202	209	Ill	191	196	188
23	258	220	—	252	264	199	239	240	218	216	Could not be contacted					
24	—	334	298	329	345	296	321	293	270	345	275	269	278	286	No sample	250
25	298	275	282	283	285	244	225	265	215	276	281	295	268	271	188	246

mined at the time of the annual physical examination of these subjects. The variation shown indicates the necessity of using caution in the interpretation of results of regimens such as those described in this paper and when the number of subjects is small.

Supplementing a diet with 400 to 900 calories from fat emulsions and expecting the subject to maintain his weight by avoiding other fats does not seem very practical. A high percentage of these individuals gained weight during the four-week experimental periods. In addition, the subjects tired of the regimens. Although the men found it easier to take three tablespoons of commercial safflower oil daily in the 1957 study than the cup of emulsified peanut oil in 1955, by the end of four weeks they were finding it difficult to take the safflower oil preparation and were tiring of substituting the calories supplied by the fat preparations for other foods. Even though all the subjects were interested in these researches and all were in various research activities themselves, it is highly doubtful that they would have continued either experimental procedure beyond the four-week duration of these studies.

If the use of unsaturated fats in the diets of hypercholesteremic patients is to be practical, it is our belief that the fats must be incorporated into the diet pattern itself. It is also important that the usual meal pattern be maintained, since food habits are extremely difficult to alter. It seems highly improbable that people like our subjects, who have a moderately elevated serum cholesterol but have suffered no myocardial infarction, would continue on a diet pattern contrary to their usual way of living for long periods of time unless easy, palatable, and very practical measures could be developed. Of even more pertinence is the doubt that a decrease in serum total cholesterol of such modest amounts is in any way effective in lessening atherogenesis or the chances of developing myocardial infarction.

It is of interest to note that the estimated average caloric intakes of the ten subjects had decreased by approximately 300 calories in 1957 as compared with 1955. Both levels are some-

what less than the 3,000 calories many physicians assume are usually consumed by the average middle-aged adult. Cholesterol intakes also decreased during this two-year period, about 130 mg, and the total fat fell from 45 per cent of the total calories to 39 per cent, though the division of fat between animal and vegetable remained the same. It would be interesting to know whether these observations represent a general trend in over-all food patterns and whether they have any relationship to the interest of the public in this subject.

Since these subjects were intellectually well above average and had participated in various "cholesterol studies" with this laboratory since 1952, it is our feeling that they may have changed their food habits to some extent.

SUMMARY

Two studies of the effect on serum lipids of the inclusion of vegetable oil preparations in the diets of adult males living at home and choosing their own diets are reported. In the first study, conducted in 1955, 20 subjects were given approximately 1,000 calories of emulsified peanut oil as a supplement to a self-selected diet designed to maintain weight for a period of four weeks. About half the subjects gained two or more pounds. There was a significant decrease ($p < 0.01$) in serum cholesterol and in the S_f 12–20 lipoprotein fraction averaging 31 and 19 mg per 100 ml, respectively.

If the subjects are arbitrarily divided into the 8 who showed less than a 10 per cent decrease in their level of serum total cholesterol and the 12 who showed more than a 10 per cent decrease, the latter showed an average decrease of 16 per cent, amounting to 48 mg cholesterol.

In 1957, 10 of these subjects took three tablespoons of commercial safflower oil preparations daily for four weeks. There was a significant decrease ($p < 0.01$) in serum cholesterol averaging 28 mg per 100 ml. When the subjects are arbitrarily divided into the 4 who showed less than a 10 per cent decrease in their level of serum total cholesterol and the 6 who showed more than a 10 per cent decrease, the latter showed an average decrease of 16 per cent, amounting to 43 mg cholesterol.

Caution must be used in the interpretation of results of regimens such as are described in this paper, since spontaneous variations in serum cholesterol are known to occur. Furthermore, it is highly questionable whether a decrease of cholesterol of this small amount from high initial levels (averaging 290 mg per 100 ml in 1955 and 264 mg per 100 ml in 1957) has any significance in decreasing atherogenesis or infarction.

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Metabolic Effect of Fat Emulsion

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DURING the past few years a great deal of effort has been expended in an attempt to develop and evaluate means of providing an adequate caloric source for parenteral feeding. One approach to this problem has been the development of fat emulsions suitable for intravenous administration to patients. The technical aspects of manufacturing oil-in-water emulsions sufficiently stable to allow prolonged storage and introduction into the blood stream have been solved to a large extent. As with other intravenous infusions, such emulsions occasionally give pyrogenic reactions. Sometimes toxic effects are noted, and there have been a few reports of what appears to be a chronic toxic effect from long, continued administration of substantial quantities of emulsion.[1–3]

The most troublesome acute reactions are chills, fever, and a peculiar type of back pain usually observed after only a few milliliters of emulsion have been given. Modification of the emulsifying agents has reduced the incidence of these acute reactions to a very low level. The emulsion manufactured by the Upjohn Company (Lipomul I.V.) has the lowest incidence of acute reactions of any currently available.

The purpose of this study was to evaluate in patients a parenteral feeding program which included protein hydrolysate, dextrose, and fat in quantities considered to be practical from the standpoint of quantity of fluid and concentration of the materials being administered. For a more adequate evaluation of the full parenteral feeding program, which included fat, protein, and carbohydrate, the effects of other regimens not including fat were tested. Also in two patients a comparison between oral and intravenous feeding programs was made.

MATERIALS

The fat emulsions used in these experiments were prepared in the Department of Nutrition, Harvard School of Public Health.[4] They contained 10 or 15 per cent cottonseed oil, 1.2 per cent soya bean phosphatide, 0.2 per cent Pluronic F68, and 4.6 per cent dextrose by weight. This emulsion is similar, but not identical, to the emulsion currently being manufactured by the Upjohn Company. A 10 per cent Amigen‡ solution was diluted with an equal volume of 10 per cent dextrose immediately before use. The exact amount of fluid that was administered to the patient was determined by weighing the containers before and after each infusion. In addition to proteins, carbohydrate, fat, and minerals shown in Table I, each of these patients received generous supplements of vitamin B complex, vitamins C, and D.

METHODS OF ANALYSIS

These studies were carried out on the hospital metabolic ward. Urine samples were collected in 24-hour periods and then pooled in 2-day lots prior to analysis. Stools, which were infrequent, were pooled and analyzed once for each 6-day period. The outputs by stool were then divided over the 6-day period in which

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‡ The Amigen was supplied by Dr. Warren Cox, Mead Johnson Company.

TABLE I

Summary of Daily Intravenous* Intake and Urinary and Stool Output—Four Subjects

Subject Case No.	Period	No. days	Av. cal/day	Av. protein intake/day (g)	Av. carbohydrate intake/day (g)	Av. fat intake/day (g)	Nitrogen (g)		Potassium (meq)		Sodium (meq)		Chloride (meq)	
							Av. intake	Av. excretion/day	Av. intake/day	Av. excretion/day	Av. intake/day	Av. excretion/day	Av. intake/day	Av. excretion/day
W. W.	I	12	742	81	100	0	13.0	12.4	73	58	60	53.2	81	76.9
Case 1	II	10	2,137	81	100	150	13.0	11.3	76	53.3	60	36.8	81	44.9
	III*	10	2,348	87.5*	146.2*	149.3	14.0	12.7	33.2	56.9	96.4	107.6	95.1	105.8
	IV	10	701	121	50	0	19.3	20.1	89.5	80	90	81.5	103	109
D. D.	I	8	907	81	140	0	13.0	13.0	73	54	60	73	162	97.4
Case 2	II	6	1,873	81	149	100	13.0	11.7	76	41	60	30	162	47
	III*	7	1,945	98*	153*	98.4	15.7	14.7	65	61	56.5	67	55	70
A. G.	I	6	2,067	85	192	100	13.6	15.7	78.5	70.0	63.9	56.5	85.0	96.0
Case 3	II	12	1,153	89	192	0	14.2	13.2	76.7	74.1	66.6	59.7	86.8	96.7
	III	12	1,304	177	141	0	28.3	26.9	112.5	104.3	132.0	112	132.0	108.0
M. M.														
Case 4	I	8	1,978	81	174.7	100	13.0	12.7	73.9	62.1	81.7	64.9	98.8	90.8

* In period III in cases 1 and 2, feeding was oral.

the samples were passed. When oral diets were used, the patients ate equal portions of the same foods each day. These diets were adjusted to correspond to the preceding intravenous intake as closely as possible by calculation. During the feeding period representative portions of the diet were homogenized and analyzed for nitrogen, potassium, sodium, and chloride. As indicated in Table I and Figures 1 and 2, the analyses gave slightly higher values than calculated, thus accounting for the variations shown. The feces and diet samples were ashed, extracted, and made up to volume with hydrochloric acid. Chloride and nitrogen were determined on the homogenized samples before ashing. Sodium and potassium were determined on a flame photometer. A micro-Kjeldahl method was used for nitrogen. All samples were run in duplicate. In a few instances when the duplicate analyses were significantly different, four additional determinations were made to arrive at the correct figure.

METHODS OF ADMINISTRATION

A standardized method of administration of fluid was employed throughout. During periods when fat, protein hydrolysate, and dextrose were given, the fat emulsion was started first, followed by the mixture of dextrose and Amigen. Except for patient M. M., all fluids were given at the rate of 5 ml per minute and started between 9:00 and 10:00 A.M. Patient M. M. had a polyethylene catheter in the inferior vena cava and the solutions were administered slowly over a 24-hour period.

PLAN OF EXPERIMENT

The principal objective of this study was to evaluate the efficacy of a parenteral feeding regimen using dextrose, protein hydrolysate, emulsified fat, minerals, and vitamins. The volumes of fluid are within the limits tolerated by most patients. In order to evaluate properly the full intravenous feeding program it was necessary to compare the results with other regimens, which included (a) periods when three of the patients received 2 liters of 5 per cent dextrose and 5 per cent Amigen; (b) periods during which two patients ate three

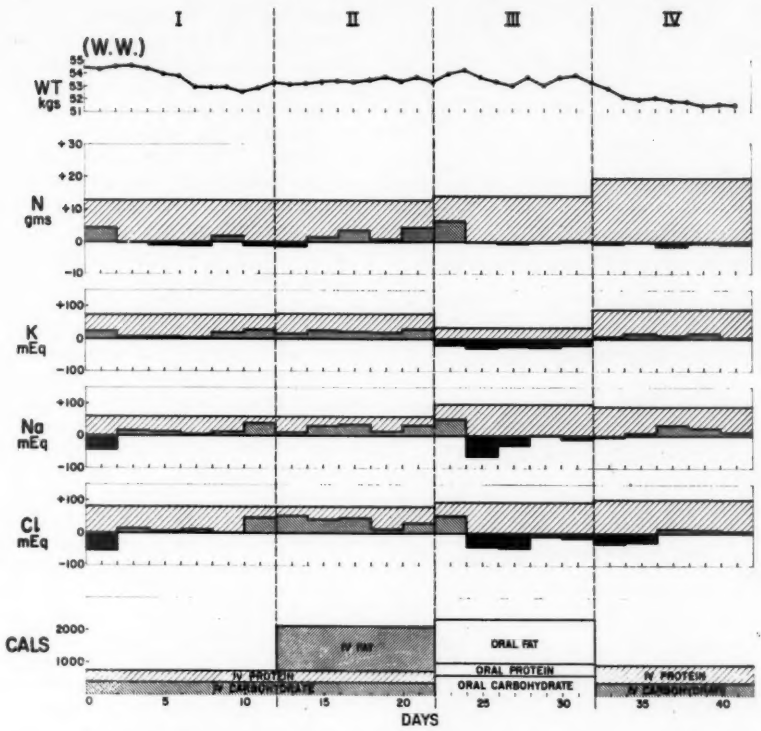

Fig. 1. Case 1, W. W. *I* and *II*. Increased nitrogen, potassium, sodium, and chloride retention during intravenous fat infusion. *III*. Oral intake. *IV*. Increased protein intake. Nitrogen, potassium, sodium, and chloride intakes are charted upward from the O line and outputs are charted downward from the top of the intake column. An excess of output over intake is shown as a solid area below the O line. A positive balance is indicated by a shaded area above the O line.

meals a day, the quantity of which was adjusted to give approximately the same amount of protein, fat, and carbohydrate as during the full intravenous feeding regimen; (c) two periods during which two patients had the protein intake increased by 50 per cent and 100 per cent. Each different regimen lasted 6 to 12 days.

Case 1. W. W.(MGH #481814) was a 26-year-old man who suffered from Friedrich's ataxia. This disease had progressed slowly since childhood so that at the time of admission there was evidence of pyramidal and spinocerebellar tract degeneration and also some cerebellar involvement. He was unable to walk and spent most of each day in a wheelchair and occupied his time with handicraft work. His admission to the hospital was for the purpose of investigating a complaint of dyspnea on exertion. He was found to have no significant heart disease, although myocarditis was considered. He volunteered to be a subject for a parenteral feeding experiment which lasted seven weeks. During five of these weeks he was fed only intravenously (Fig. 1). He suffered no ill effects of this rather strenuous program. He was very hungry during the first three days of each intravenous feeding period. This hunger was accentuated approximately three hours following the infusion of dextrose-Amigen solution presumably because of fluctuation of blood sugar concentration.

Case 2. D. D.(MGH #873507) was a 48-year-old man who entered the hospital for a hip arthroplasty necessitated by degeneration of the hip joint secondary to trauma. In November, 1954, this operation was carried out. While on the rehabilitation ward five months later he developed acute cholecystitis and underwent a cholecystectomy. Convalescence from this operation was uneventful. One month later he was transferred to the metabolic ward as a volunteer subject for a parenteral feeding experiment. He was fed intravenously for 14 days (Fig. 2; Table I), and this was followed by a seven-day period of oral intake providing approximately

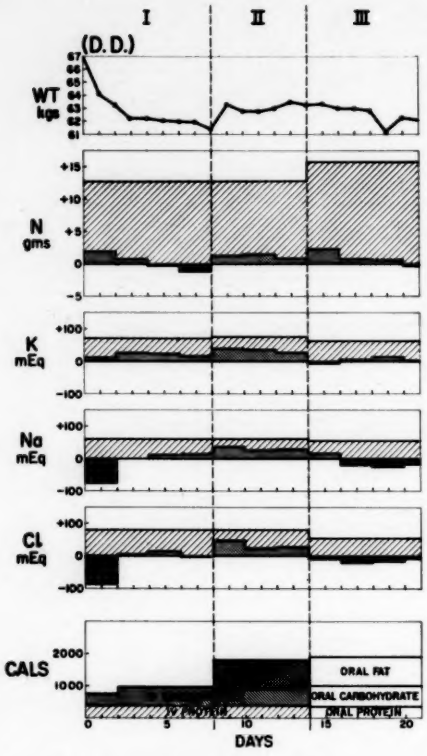

Fig. 2. Case 2, D. D. *I.* Increasing nitrogen excretion on calorie-deficient regimen. *II.* Restoration of positive balance on addition of fat and retention of sodium and chloride. *III.* Oral intake for comparison with intravenous period. Excretion of sodium and chloride from period II.

the same amount of protein, carbohydrate, and fat. Throughout the whole experiment he was in bed except for brief periods of sitting in a wheelchair and gait training in a walker each day. He remained well throughout the experiment and rarely complained of hunger during the two weeks of intravenous feeding.

Case 3. A. G.(MGH #743224) was a 63-year-old man who was admitted to the hospital for treatment of carcinoma of the esophagus. Two years previously he had had electrocoagulation of a carcinoma of the floor of the mouth followed by a radical neck dissection. The esophageal lesion had caused dysphagia to the extent that he could not eat solid foods, but he took liberal quantities of high-caloric liquids including 1 pint of whiskey each day. He had lost 15 pounds of body weight in the two years prior to admission, but was well nourished, with normal body fat. The lesion was considered inoperable, and a course of x-ray therapy was begun. He was a chronic alcoholic, but the only flocculation test. He remained in the hospital only on the condition that he be supplied with whiskey "for his nerves." Four days after beginning x-ray treatment he was started on parenteral feeding. The feeding program and the results are shown in Table I and Figure 3. The calories shown as alcohol were supplied by mouth as a 90-proof, commercial brand of whiskey. Throughout the period of observation he ran a low-grade fever (100 to 101° F.) and felt poorly. The fever started the day x-ray treatment was begun.

Case 4. M. M.(MGH #768575) was a 56-year-old woman with chronic heart disease. An embolus of the superior mesenteric artery necessitated the resection of her entire small bowel distal to the second portion of the duodenum and right half of her colon. A duodenocolostomy was performed. Her immediate convalescence proceeded uneventfully, but she was never able to retain food or liquid in her stomach. Vomiting and watery diarrhea continued steadily even when nothing was taken by mouth. She lost weight rapidly. Approximately four weeks after operation a daily intravenous feeding program of 1,000 ml of 10 per cent dextrose and water, 1,000 ml of 10 per cent fat emulsion, 1,000 ml of 10 per cent Amigen, and 500 ml of 5 per cent dextrose and saline was begun. In addition she received 40 meq of potassium chloride and generous vitamin supplements in each daily ration. After one week of this daily feeding program the balance study summarized in Figure 4 was carried out. This was continued for eight days, and terminated when the patient had an aortic embolus. An embolectomy was performed, but the patient died a week later of infection originating from a localized right gutter abscess which had been present since her original operation. The results of the eight-day balance study are shown in Table I and Figure 4.

DISCUSSION

The balance studies carried out in these four patients supplement those previously reported from this department by VanItallie, Moore, Geyer, and Stare[5] and allow further examination of some of the many aspects of the problem of parenteral feeding. Three of these subjects were well nourished at the start of the experiment. In patients W. W. (Case 1) and D. D. (Case 2) initial periods of moderate caloric restriction lasting 12 and 8 days, respectively, undoubtedly had some influence upon the result obtained when a caloric supplementation by intravenous fat was given. It seems reasonably clear that emulsified fat supplied under these circumstances will promote nitrogen retention. Note should be made of the fact that the caloric intake was about 30 calories per kg per day and nitrogen intake was 13 g per day.

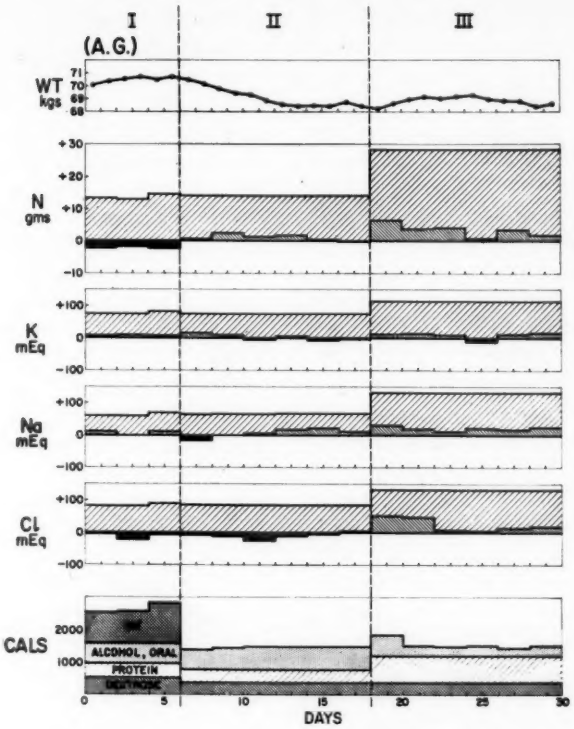

Fig. 3. Case 3, A. G. *I.* Negative nitrogen balance while receiving intravenous fat. *II.* Approximate equilibrium on calorie-deficient regimen. *III.* Positive balance gradually returning to equilibrium on high-protein intake.

The results of these two experiments are in conformity with the results of other experiments designed to test the relationship between caloric intake and nitrogen metabolism.[6–14] The control periods (*I*) in these same two subjects illustrate another aspect of the overall problem of parenteral nutrition and the relationship between caloric supply and nitrogen retention. On a caloric intake of less than 1,000 calories per day there was only slight negative nitrogen balance, and it is obvious from the weight records that the deficit in calories was being made up from fat stores. The temporary nature of this adjustment is shown by the increasing negativity of the nitrogen balance. It appears that caloric supplementation with intravenous fat will reverse this trend and that maintenance at this level of calorie and nitrogen intake will sustain posi-

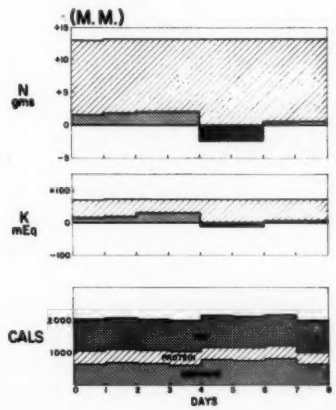

Fig. 4. Case 4, M. M. Eight-day balance study carried out on seriously ill patient five weeks after resection of entire small bowel and right colon, and one week after beginning the intravenous feeding program, which continued through the study period.

tive balance, or at least equilibrium, for 6 to 10 days (and probably longer, as discussed below).

The substitution of the oral method for intravenous feeding demonstrated no detectable difference between whole food protein per os and protein hydrolysate administered intravenously. Further, emulsified vegetable fat (cottonseed oil) introduced directly into a systemic vein appears to be equivalent to mixed animal and vegetable fat taken orally. The rather widely held clinical impression that the oral route of feeding is advantageous was not borne out by the results of these two comparisons.

The experiment carried out on patient A. G. (Case 3) was considerably different from the others. There was no initial period of caloric restriction, and a moderate dose of whiskey was taken orally throughout the experiment. The patient was a chronic alcoholic with some hepatic impairment and he was receiving radiation therapy. Despite these qualifying factors certain conclusions are obvious. First, the provision of what was thought to be adequate calories and protein did not insure nitrogen retention or even equilibrium. Second, the prompt change to positive nitrogen balance after the fat infusion was stopped indicates that, in this patient, fat infusion was a detriment as far as maintaining nitrogen equilibrium was concerned. Whether this was due to unrecognized toxic reaction to the emulsion or failure to metabolize the extra fat because of pre-existing hepatic impairment is not known. The facts that a nitrogen deficit of 2 g per day was associated with fat administration and that positive balance promptly ensued when the fat was stopped are indicative of something more than only failure to metabolize the additional fat.

For this patient the optimal intravenous feeding regimen was a high-protein, fat-free intake which resulted in nitrogen retention even at submaintenance caloric levels. Further study of the metabolic effect of emulsified fat administration to patients with hepatic impairment is needed but, pending this, some reservations must be held concerning the utilization of fat by such patients.

The eight-day balance study carried out on patient M. M. (Case 4) illustrates one additional point—that a very ill patient can be maintained in nitrogen equilibrium for prolonged periods by intravenous feeding with protein hydrolysate, dextrose, and emulsified fat. This balance study was carried out five weeks after resection of almost all of this patient's intestine. We have no explanation for the two-day period of negative balance interposed between periods of slight positive balance, but it seems reasonably certain that in general she was in equilibrium and not wasting body stores of protein.

It will be noted in Table I that patients W. W. and D. D. showed a definite retention of sodium and chloride during periods when fat was being administered intravenously. Subsequent oral feeding periods were accompanied by excretion of sodium and chloride in excess of intake. Fat administration therefore appears to have been responsible for unusual sodium retention. The significance of this finding is not clear. On the basis of present information there is little to indicate adrenal stimulation as a result of fat administration. It seems possible that the introduction of a relatively large quantity of surface-active phospholipid used as emulsifiers might affect membrane permeability throughout the body. For short periods of fat administration and when sodium intake is low, this effect does not appear to be a serious limitation to the use of emulsified fat, but it bears consideration in patients with limited cardiac reserve and during longer periods of fat administration, particularly if salt is being given concurrently. Further study of this effect of fat emulsions is obviously needed.

SUMMARY

The effect of intravenous fat emulsion on nitrogen, potassium, sodium, and chloride metabolism was studied in four subjects, using the balance technic. It is concluded that fat emulsion promotes nitrogen and potassium retention in some patients, but that in other patients administration of fat emulsion under similar circumstances may be associated with excess nitrogen losses. Comparison of oral

and intravenous feeding failed to demonstrate any difference in nitrogen metabolism, and it is concluded that the two methods are equivalent. Attention is directed to the fact that sodium and chloride retention may be associated with administration of intravenous fat.

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Comparison of Aortic Atherosclerosis in the United States, Japan, and Guatemala

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THE study of geographic variations in the prevalence of coronary artery disease, as a measure of atherosclerosis, has provided information which suggests a means of controlling the disorder. As summarized by Keys,[1] the incidence of vascular heart disease is apparently related to the fat content of the diet by virtue of the latter's influence upon the level of serum cholesterol. This is the explanation he has given for the infrequency of coronary artery disease in groups whose dietary fat content is low, e.g. the Bantu in South Africa, the Japanese residents of Kyushu, and the natives of Madrid and Naples. By contrast, the high fat content of the diet seems to be related to the prevalence of coronary disease in the United States, Denmark, Sweden, and England. The distinction is neither a national nor racial characteristic, since it is lost in groups who customarily consume more fat, such as bankers in Naples, Italians residing in Bologna (or the United States), or Japanese residents of Hawaii.

The vital statistics which permit these conclusions, impressive as they may be, are still an indirect measure of an anatomically demonstrable process. Accordingly, autopsy verification is required to establish confidence in their validity. The studies of Higginson and Pepler[2] upon the South African Bantu and those of Kimura[3] upon the Japanese in Kyushu are pertinent but suffer from a shortcoming which has been common to virtually all pathologic descriptions of atherosclerosis. Until recently there has been no satisfactory objective way of recording the degree of atherosclerosis observed at autopsy. Unavoidably, except for occlusive lesions, there has been considerable inaccuracy in comparing the data of different observers, especially when there has not been a common background of experience.

Accordingly, it has been considered appropriate to extend the use of a more objective appraisal technic[4] to a series of consecutive autopsies performed in Sapporo, Japan, for comparison with similarly evaluated unselected postmortem material from Guatemala, New Orleans, and Los Angeles. The Guatemalan and New Orleans data have been reported previously[5] and subsequently have been compared with a representative autopsy sample from Costa Rica.[6]

These studies disclosed that there were no discernible differences in atherosclerosis before the age of 30, by which time virtually all individuals display some degree of intimal disease. Thereafter, differences in the rate of progression characterized the disease process in the three countries. As measured both by extent of surface involvement and by the character of the lesions, atherosclerosis advanced more rapidly in New Orleans than it did in Guatemala or Costa Rica. Of the three countries, Guatemala displayed the lowest incidence of severe disease. Corresponding differences had been described[7] in dietary fat, serum cholesterol, and S_f 0–12 lipoprotein levels. A similar study comparing atherosclerosis in Los Angeles and India (Vellore)[8] demonstrated less extensive and less severe arterial disease in the latter.

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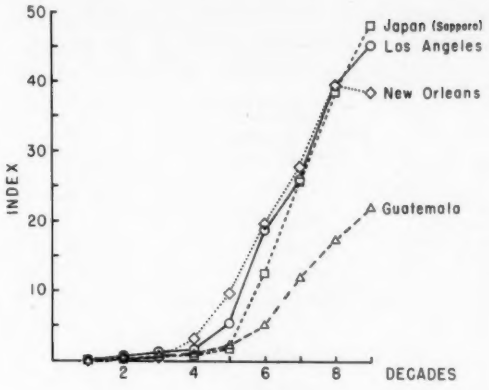
MATERIAL AND METHODS

The degree of atherosclerosis was appraised in the unstained aortas of 659 consecutive autopsies performed at the Los Angeles County Hospital and in 260 consecutive postmortem examinations at the University Hospital in Sapporo, Japan. Males and females were included in both groups, but have been combined in tabulating the number of individuals in each decade listed in Figure 1.

As previously described,[4] the appraisal technic considers both the extent of surface involvement and the character of the lesions observed. Intimal lesions are classified as: grade 1, fatty streaks; grade 2, fibrous and atheromatous plaques; grade 3, necrotic, hemorrhagic, or thrombotic plaques; and grade 4, calcified plaques. In terms of surface area involvement the five groups were: group 0, less than 5 per cent surface involvement; group A, 6 to 15 per cent involvement; group B, 16 to 33 per cent surface involvement; group C, 34 to 50 per cent surface involvement; and group D, more than 51 per cent surface involvement. The findings are listed as an "atherosclerotic profile," a five-digit figure that expresses the proportion of the intima that is diseased and the decimal fraction of the diseased portion constituted by each of the four types of lesion. By weighting, arithmetically for surface extent and logarithmically for the grades of lesion, an atherosclerotic index is derived expressing severity on a scale ranging from 0 to 100.

RESULTS

The findings in Japan and Los Angeles are presented graphically in the accompanying figures. For purpose of comparison the New Orleans and Guatemala data[5] have been plotted as well. There is a progressive rise of the atherosclerotic index (Fig. 1) with age in all localities. Prior to 30 years, the intimal process is identical in the four areas. Subsequently, severity progresses most rapidly in the two United States groups and least rapidly in Guatemala. The statistical significance of this difference has been previously established.[5] The Japanese figures indicate a relative lag in the accretion of intimal disease



CASES

Decade	1	2	3	4	5	6	7	8	9	Total
New Orleans	158	68	74	73	52	65	69	30	27	616
Los Angeles	5	10	9	24	51	98	161	185	116	659
Japan	37	12	25	39	37	45	28	24	13	260
Guatemala	31	20	39	62	50	47	44	22	9	324

Fig. 1. Atherosclerotic index, aorta.

during the fifth and sixth decades, but thereafter the severity of aortic atherosclerosis in Sapporo coincided with that found in New Orleans and Los Angeles. Statistically the differences between Sapporo and New Orleans in the fifth and sixth decades are significant. However, the intermediate position of the Los Angeles figures in the fifth decade cannot permit us to consider this finding as more than suggestive.

The surface extent of aortic involvement is depicted in Figure 2 for the first, third, fifth, and seventh decades. With aging, there is a progressive increase of the proportion of cases displaying maximal surface involvement (group D). This occurs at a slower rate in Sapporo than in the two United States cities and is most evident in the fifth decade; but, as the graph of the seventh decade discloses, the process is slower still in Guatemala.

Figure 3 and Table I demonstrate the relative proportions of the four grades of atherosclerotic lesions. It is to be noted that, following the widely accepted concept of atherogenesis, grade 1 lesions (lipid streaks) predominate in the early decades and are succeeded by a growing proportion of fibrous plaques (grade 2). The complicated, ulcerated, or calcified lesions (grades 3 and 4) appear in the fourth decade in both New Orleans and

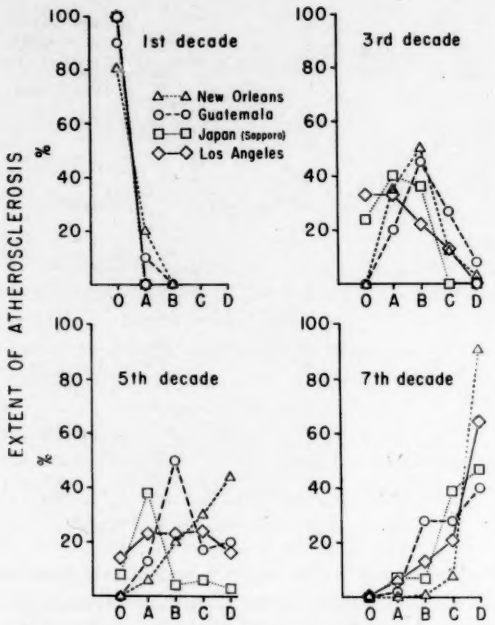


Fig. 2. Aortic atherosclerosis, by area involved.

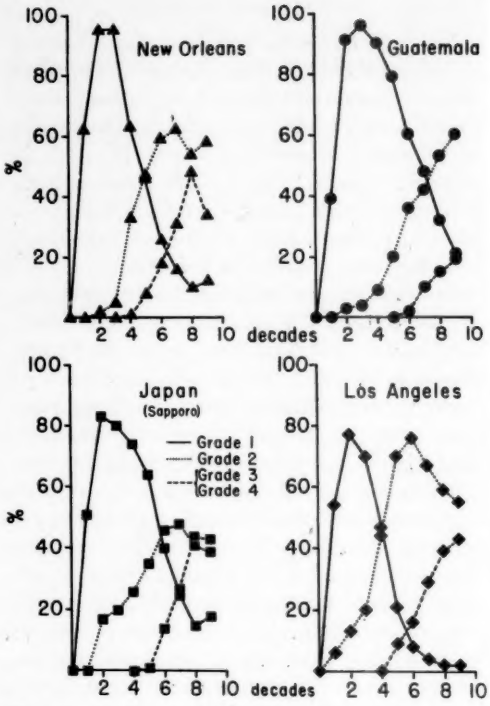


Fig. 3. Aortic atherosclerosis. Type of lesion by decade.

Los Angeles and in the fifth decade in Japan and Guatemala. In Guatemala the progressive increase of grade 3 and 4 lesions is very slow, whereas in Japan they rapidly attain the magnitude observed in the United States material. It is to be noted that the proportion of grade 1 lesions, in the early decades at least, is higher in New Orleans and Guatemala than in Los Angeles and Sapporo. This, however, is only a technical rather than a true distinction and reflects only the accentuation of lipid lesions produced by staining.

DISCUSSION

In view of the comparatively low incidence of fatal coronary artery disease in Japan[9–11] and the relatively low levels of serum choles-

TABLE I

Relative Proportion of Atherosclerotic Lesions (Average Profile)

Decade	New Orleans Grade*				Los Angeles Grade*				Japan Grade*				Guatemala Grade*			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
1	62	0	0	0	54	6	0	0	51	0	0	0	39	0	0	0
2	95	2	0	0	77	13	0	0	83	17	0	0	91	3	0	0
3	95	5	0	0	70	20	0	0	80	20	0	0	96	4	0	0
4	63	33	0	1	47	44	0	0	74	26	0	0	90	9	0	0
5	46	47	3	5	21	70	2	7	64	35	1	0	79	20	0	0
6	26	59	8	10	8	76	4	12	40	46	8	6	60	36	0	2
7	16	62	13	18	4	67	9	20	25	48	14	13	48	42	4	6
8	10	54	21	27	2	59	9	30	15	41	18	26	32	53	6	9
9 and over	12	58	13	21	2	55	8	35	18	39	21	22	21	60	7	12

* See text for description of grading.

terol[12] among the Japanese, it is surprising not to find more striking differences in the aorta between the United States and Japan. Several possibilities may be suggested:

(1) Major differences may be hidden by individual variation in the application of the appraisal technic. It would indeed be more accurate to have one person assay all the aortas from each locale. Since this is not usually possible it is pertinent to note the compliance of the Los Angeles with the New Orleans data and to suggest that it is practical to compare atherosclerotic data obtained by different observers.

(2) It must be established that this relatively small Japanese sample is representative of the general populace and not biased by the inclusion of numbers of more prosperous individuals with an entirely different, non-average, dietary.

(3) It has been demonstrated that coronary atherosclerosis is generally less severe than aortic atherosclerosis, but parallels the process in the aorta.[8,13] If this usual disparity were even greater among the Japanese, it would explain a low incidence of vascular heart disease despite severe aortic sclerosis. It is pertinent to note that there were only 10 cases of myocardial infarction or coronary occlusion among 147 Japanese older than 40 years, whereas these were found in 150 of 611 similarly aged Los Angeles residents.

(4) Serious coronary artery disease may not be due to atherosclerosis, per se, but to a complication of it. This, happening less frequently in Japan, would explain the apparent discrepancy.

(5) Sapporo may not be representative of Japan as a whole in regard to atherosclerosis and the factors which contribute to it.

Obviously further investigation is needed to assay these possibilities for the truth.

SUMMARY

Some degree of atherosclerosis is present in virtually all individuals by the thirtieth year in New Orleans, Los Angeles, Japan (Sapporo), and Guatemala.

Subsequent aging is associated with a progressive increase of aortic atherosclerosis. The rate of increase is least in Guatemala and greatest in the United States. Within the limits of the assay procedure atherosclerosis is identical in Los Angeles and New Orleans.

There is suggestive, but not conclusive, evidence that in Japan the rapid increase in the severity of atherosclerosis that characterizes the middle decades in the United States is delayed. In the latter decades, however, the intimal disease, in this sample at least, is fully as severe in Japan as it is in New Orleans and Los Angeles.

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Errata

In the article "The Metabolism of Plasma Unesterified Fatty Acid," in the November-December issue (Vol. 6, No. 6, pp. 669–680) there are two errors:

Page 670, first column, third line. *1 mg of glucagon* should read: "1 unit glucagon."

Page 677. Equation 1 should read: $\bar{\nu}_A = \Sigma \nu_{Ai} = \sum_i \frac{n_i k'_{Ai} c_A}{1 + k_{Ai}' c_A}$ Equation 1.

Caloric Intake in Relation to Energy Output of Obese and Non-Obese Adolescent Boys

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A DECREASED tendency for muscular activity was observed by Rony[1] to be a common finding among many obese persons. He also noted that as weight increases, the impulse for physical exertion decreases. This results in a decreased energy requirement in spite of the increase in weight and caloric intake continues to be in excess of need. Bruch[2] called attention to the relative inactivity of many obese children; that of obese women also has recently been noted.[3]

It has been demonstrated that when activity is reduced to below a minimum in experimental animals[4] and in adult men[5] a corresponding decrease in food consumption does not result and obesity develops. Genetically obese mice are characterized by their inactivity.[6] Conversely, exercising animals with different types of obesity causes spontaneous weight loss.[4,6] In these studies lack of activity appeared to be not only a consequence but a possible causal factor in obesity.

Porter[7] in 1920, and later others[8,9] observed the tendency for greater weight increases in children to occur during the fall and winter months than during the spring and summer. Johnson, Burke, and Mayer[10] found that the onset of excessive weight gain among obese children in the public schools of Newton and Brookline, Mass., generally occurred during the winter and suggested that inactivity may be an important factor in the development of obesity in adolescence. A comparison of food intakes and activity schedules of 28 obese high school girls selected from this population with controls of normal weight and of the same height, age, school grades, and socioeconomic status[11] showed that these obese girls ate less, not more, than their normal weight controls, but were strikingly less active physically. Underexercising rather than overeating appeared to be distinctive of this obese group of adolescent girls.

This paper reports the average daily caloric intake and physical activity of 14 obese and 14 paired control non-obese adolescent boys. Comparisons between the obese and non-obese groups were made for the school year and for 8 weeks at a summer camp. Heights and weights and the average daily food intakes of a larger group of 65 boys, 13 to 15 years of age, are presented so as to better situate the sample.

METHODS

(1) *Description of Population:* Sixty-five adolescent boys attending a private camp for boys in central Maine for eight weeks during the summer of 1957 participated in the study. The majority of the boys resided in the Northeast region of the United States. Ages were recorded to the nearest birthday. Heights and weights were taken without shoes and clothing and were recorded at the beginning and end of the study. The mean, standard deviation and range of the ages, and of the initial heights and weights are presented in Table I.

The heights and weights of this group of adolescent boys were definitely above the

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TABLE I

Age, Height, and Weight of 13- to 15-Year-Old Boys Living in Northeast United States (Standard: Stuart-Meredith 50th Percentile)[23]

Age group (years)	Number	Age (years)	Height (inches)	Weight (lb)	Standard ht. (inches)	Standard wt. (lb)
13	29					
Mean		13.6	65	124.2	61.0	93.0
S. D.		0.3	3.1	20.2		
Range		13.1–13.9	59.75–70.25	88–156		
14	29					
Mean		14.4	66	124.8	64.1	107.6
S. D.		0.3	3.1	2.1		
Range		14–14.9	49.25–71	85–169		
15	7					
Mean		15.4	67.5	135.1	66.1	120.1
S. D.		0.2	1.5	26.7		
Range		15–15.8	63.25–71.5	96–188		
Total group	65					
Mean		14.2	65.7	134.7		
S. D.		0.6	3.1	21.6		

S. D. = Standard deviation.

Stuart-Meredith 50th percentile figures for their age (Table I). The 13-year-olds were in the 90th percentile; the 14- and 15-year-olds were closer to the 75th percentile. A comparison of the measurements obtained in this group with others previously obtained for adolescents of the same age is of interest. Studies conducted between 1928 and 1953 on 13- to 15-year-old boys in the Northeast region of the United States are summarized in a reference handbook of the United States Department of Agriculture prepared by Hathaway.[12] The 13-year-old mean height in the group studied here is 4.3 inches taller and the mean weight 26.3 pounds heavier than the mean of 13 previous studies of 13 to 711 boys. The 14-year-old mean height is 3.1 inches taller and the mean weight 16.2 pounds heavier than the mean of 13 previous studies reporting data on 7 to 711 boys. The 15-year-old mean height is 2.1 inches taller and the mean weight 15.1 pounds greater than the mean of 12 previous studies reporting measurements on 6 to 707 cases.

(2) *Maturation:* Greulich ratings were determined as an index of sexual maturation.[13] The ratings are as follows:

1. Immature, pre-puberal boy
2. (a) Noticeable growth of genitalia
   (b) Beginning of growth of pubic hair
3. (a) Further growth of genitalia and pubic hair
   (b) Beginning axillary and facial hair (upper)
4. (a) Further growth of genitalia and pubic hair (more adult type)
   (b) Axillary and facial hair more generalized and heavier
5. Adult male

(3) *Skinfold Measurements:* Skinfold thickness measurements were made with a constant pressure caliper having a contact surface of 40 mm^2 designed at the Laboratory of Physiological Hygiene, Minneapolis. The skinfold measurements were made at the following sites:

(A) Arm, at a mid-posterior point half way between the acromion (acromial process of the scapula) and the tip of the elbow. The forearm was held in 90° flexion and the fold was held parallel to the long axis of the arm.

(B) Scapula, below the tip of the scapula. The skinfold was lifted to form a 45° angle from the tip of the scapula to the spine.

(C) Abdomen, at the mid-axillary line midway between the ribs and the iliac crest. The skinfold was lifted parallel to the mid-axillary line.

Measurements of both sides of the body were averaged for each of the three described sites.

TABLE II

Weight, Skinfold Thickness, and Greulich Rating of Obese Adolescent Boys and Paired Non-Obese Controls

Non-obese	Weight (lb)	Skinfold thickness (mm)			Maturation (Greulich rating)	Obese	Weight (lb)	Skinfold thickness (mm)			Maturation (Greulich rating)
		Scapula	Arm	Abdomen				Scapula	Arm	Abdomen	
1	138	12.0	13.0	8.5	4	1	132	19.5	16.75	21.25	4
2	132	7.5	11.0	8.0	4	2	124	18.0	22.5	10.0	2
3	95	8.5	8.75	6.5	3	3	120	11.5	18.5	18.0	2.5
4	102	6.0	10.5	6.5	2.5	4	120	13.75	16.0	14.0	2.5
5	104	6.0	5.5	4.5	3.5	5	156	14.5	18.0	14.0	4
6	127	11.0	15.0	8.0	3.5	6	136	23.0	21.0	32.0	2
7	96	5.75	8.75	6.75	3.0	7	135	18.0	24.5	21.5	3.5
8	142	7.25	8.25	10.0	4	8	188	25.0	22.0	36.5	4.5
9	138	7.0	8.0	4.5	4	9	169	19.0	19.0	19.5	4
10	108	7.0	13.5	13.75	3	10	134	14.25	15.0	14.0	3
11	88	6.75	10.5	5.5	2.5	11	104	15.0	13.75	13.0	1.5
12	126	6.0	5.5	4.75	4	12	143	15.0	14.5	22.5	3.5
13	127	8.75	8.5	6.5	4	13	150	15.0	17.0	13.25	4.5
14	137	9.0	13.0	10.0	4.5	14	128	15.25	15.25	15.25	3
Mean weight: 118.6 ± 18.9 lb.*						Mean weight: 138.5 ± 21.7 lb.*					
Mean height: 65.6 ± 3.5 in.						Mean height: 65.5 ± 2.8 in.					
Mean age: 14.1 ± 1.0 yr.						Mean age: 14.2 ± 1.2 yr.					
* Mean ± standard deviation.						* Mean ± standard deviation.					

(4) *Sample:* It is not uncommon to define obesity on the basis of relative weight, especially when only heights and weights are available, as is the case for surveys made from school records involving large populations.[10] Keys[14] and others[15,16] have shown that the classification of individuals into obese and non-obese on the basis of proportion of body weight to a "standard" or "ideal" weight for sex, age, and height is far less reliable than methods based on body composition. This is particularly true in adolescence, when wide variations in linear growth and body composition are so common. Inasmuch as a large fraction of body fat is deposited as subcutaneous adipose tissue, the thickness of skinfolds is a more direct measure, as well as a quantitative index, of leanness and fatness.[16]

Consequently, the boys were classified as obese and non-obese according to the combined skinfold thickness measurements of the arm, scapula and abdomen. An arbitrary figure of 40 mm and over was used as the lower limit defining obesity. Fourteen boys, or 21.6 per cent, had skinfold thicknesses ranging from 42 to 84 mm and were termed "obese." Fifty-one boys had measurements ranging from 14 to 35 mm and were categorized as "non-obese." In this paper obesity and non-obesity are defined according to this arbitrary standard. In some instances, these classifications probably would not coincide with those obtained by the current clinical technic of observation, i.e., some of the obese boys did not appear to be "obese."

The fourteen obese boys were paired with control non-obese boys of the same age, height, and maturation. The weights, skinfold thickness measurements and Greulich ratings for these two groups are given in Table II.

(5) *Dietary Records:* The average daily food intake for the school year was estimated for all the boys at the beginning of the camp season from nutritional histories obtained by the research interview method of Burke.[17,18] From a second dietary interview with the "obese" and control "non-obese" groups in the final week of the camp season the average daily food intake during eight weeks of camp life was estimated. This latter information was supplemented with food records of actual intakes for several boys. These served as checks for the camp interviews. The daily food records are not reported as they were similar to the av-

TABLE III

Classification of Occupations According to Energy Expenditure for a 60 to 70 kg Man

Light *Under 150 cal/hr*	Moderate *150–250 cal/hr*	Very active *Over 250 cal/hr*
No muscular activity	Walking activities	Hiking with pack
Sleeping	Slowly (2 mph)	Heavy domestic chores
"Relaxing"	Moderately fast (3 mph)	Mowing lawn
Listening to radio	Canoeing	Shoveling snow
Watching television		Baseball
Light sedentary activities		Bicycling
Eating		Bowling
Fishing		Dancing
Playing piano, horn, woodwind		Football
School classes		Golf
School homework		Gymnastics
Spectator sports		Hockey
Light domestic chores		Horseback riding, trot
Dishwashing		Rowing
Making bed		Swimming
Standing activities		Table tennis
Riflery		Tennis
Washing, dressing		Track
Standing at ease		Wrestling

erage intakes estimated by the dietary interview.

(6) *Activity Records:* Occupations engaged in by the obese and non-obese boys were separated into three general groups graded according to the approximate rate of average energy expenditure if performed by a 60 to 70 kg man.[19] These were: *light*, utilizing 150 cal/hour or less; *moderate*, utilizing 150 to 250 cal/hour; and *very active*, utilizing over 250 cal/hour. Table III gives examples of the type of exercise in each of the three categories. No actual determination of individual energy expenditure was conducted. This method was used to arrive at a semiquantitative activity index rather than at an estimate of the individual energy expenditure in calories.

Twenty-four-hour activity schedules typifying school day and weekend activities were obtained by recall for the obese and non-obese control groups at the beginning of the camp season and are reported as estimated hours per day for the three levels of exercise. The camp activity schedules were obtained by two procedures: (a) At the end of the camp season the duration and degree of participation in two daily supervised free-choice camp activity periods were obtained from camp records submitted routinely every week for six weeks by each boy's counselor. Generally, one counselor supervised seven or eight campers. The time spent for activities corresponding to the three levels of exercise was expressed as a percentage of the total number of recorded periods during the six weeks. (b) The boys were interviewed for information on additional non-recorded activities engaged in, whether supervised or not. At this time, an attempt was made to ascertain their attitudes toward all camp activities. The hours per day spent in very active exercise alone at camp was estimated by combining the information recorded by the counselor and that obtained by the interviewer.

RESULTS

(1) *Daily Caloric Intake*

(A) *School Year:* The average daily intake of the 65 boys during the school year was 3380 ± 854 cals, comprising 144 ± 30 g of protein and 185 ± 40 g of fat. Table IV lists the dietary information for the 14 non-obese control boys and the 14 obese boys. The 14 controls had a mean daily food intake of 3476 ±

TABLE IV

Average Daily Food Intakes During the School Year of Obese Adolescent Boys and Paired Non-Obese Controls Obtained by Research Dietary Interview

	Number	Calories	Protein (g)	Fat (g)
Non-obese group	14			
Mean		3,476	129	174
S. D.		625.8	24.4	32.0
Range		2,680–4,815	101–182	134–236
Obese group	14			
Mean		3,011	111	146
S. D.		580.0	18.0	30.6
Range		2,275–3,865	92–139	95–189

S. D. = standard deviation.

626 cal, 129 ± 24 g of protein and 174 ± 32 g of fat. Thus, of the total calories of the controls, 15 per cent were protein and 45 per cent were fat. The 14 obese boys consumed an average of 3,011 ± 580 cal daily; 111 ± 18 g of protein and 146 ± 31 g of fat. Thus for the obese boys, too, 15 per cent of the calories were contributed by protein and 44 per cent by fat. The mean daily average food consumption of the non-obese group was 465 calories greater than that of the obese group, a statistically significant difference ($p < .02$ by the paired "t" test).

(B) *Summer Camp:* The 14 controls had a mean daily food intake in the summer camp of 4628 ± 81[illegible] cal, 179 ± 37 g of protein and 223 ± 47 g of fat. Protein supplied 15 per cent of the total calories, and fat 43 per cent. A mean daily caloric intake of 3430 ± 617 cal containing 134 ± 28 g of protein and 166 ± 37 g of fat was obtained from the camp dietary histories for 13 obese boys (one boy left the camp at mid-season). Protein supplied 16 per cent of the average total calories; fat supplied 44 per cent. There was a significant ($p < .01$) mean difference of 1,227 cal less for the 13 obese boys than for their non-obese controls. The information obtained from the camp dietary histories is summarized in Table V.

(C) *Comparison of Intakes During School Year and at Camp:* The mean caloric intake for the 14 controls at camp was significantly greater than that estimated from their home dietary histories ($p < .01$) This was of borderline significance for the group of 13 obese boys ($p \simeq .05$). All of the non-obese control boys consumed more calories during the camp season than their daily average intake estimated for the previous school term. The increase in caloric intakes at camp ranged from 200 to 2,295 cal with a mean of 1152 ± 367 cal. By contrast, four of the 13 obese boys had intakes at camp smaller than those characteristic of the school year. For these, the reduction in food intake ranged from 40 to 295 cals daily. The increase in food intake for the 9 other obese boys ranged from 30 to 1,770 cal daily. The mean increase for this group was only 472 ± 219 cal.

TABLE V

Average Daily Food Intakes During Eight Weeks at a Summer Camp of Obese Adolescent Boys and Paired Non-Obese Controls Obtained by Research Dietary Interview

	Number	Calories	Protein (g)	Fat (g)
Non-obese group	14			
Mean		4,628	179	223
S. D.		810.9	37.4	46.9
Range		3,580–4,965	127–278	148–317
Obese group	13			
Mean		3,430	134	166
S. D.		617.2	27.8	36.8
Range		2,295–4,605	84–175	100–228

S. D. = standard deviation.

(2) *Daily Activity*

(A) *School Year:* Little difference was found between the means of the control and obese groups of the estimated number of hours per day spent in light, moderate, and very active exercise as obtained from home activity schedules (Table VI).

TABLE VI

School Year Activity (Hours per Day)

	Light	Moderate	Very active
Group mean	Under 150 cal/hr	150–250 cal/hr	Over 250 cal/hr
Non-obese	21.1	1.2	1.7
Obese	20.7	1.4	1.9

(B) *Camp:* The main percentage of supervised activity periods at camp devoted to the light, moderate, and very active exercise was about the same for the obese group as for the paired controls (Table VII).

The estimated average number of hours per day devoted to active sports at camp out of a

TABLE VII

Supervised Camp Activity Periods (Per Cent)

	Light	Moderate	Very active
Group mean	Under 150 cal/hr	150–250 cal/hr	Over 250 cal/hr
Non-obese	43	9	48
Obese	43	7	50

possible average maximum of 5.5 hours was 3.2 hours for the obese and 2.9 hours for the non-obese controls. This represents a 68 per cent increase in very active exercise for the obese and a 71 per cent increment for the controls over the customary time spent in active sports during the school year. Eight obese boys spent an average of 0.6 hour per day less in very active exercise at camp than their controls; five obese boys spent an average of 1.4 hours more than their controls.

The head counselor rated the boys according to the type of activity which each preferred. A rating of "A" indicated a preference for activities that involve running; "B" for activities that involve some running, and "C," little or no running. This rating expressed to some extent the relative amount of energy the boy was apt to expend even when he did join an active sports group. For example, a boy might have "gone out" for tennis, but could have been usually observed to not enter into the sport vigorously. The ratings were as shown in Table VIII.

TABLE VIII
Types of Activity Preferred by Obese and Non-Obese Control Boys

Rating of preferred activity	Number	
	Control	Obese
A (running activities)	2	1
B (activities with some running)	6	2
C (sedentary)	6	10

The observed frequencies in the distributions of the three ratings were not statistically significant on the hypothesis that equal proportions would be found for the obese and the non-obese. The ratings do, however, show a trend which is of interest.

(3) *Weight Record*

The mean weight of the obese group at the beginning of the camp season was equal to that at the end of eight weeks. By contrast, there was a 3.1 per cent average increase in weight in the control group. Eight obese boys who devoted less time daily to very active exercise than their controls showed no weight change. Their controls gained an average of 3.6 per cent in weight. The obese boys who devoted more time to very active exercise than their controls had a reduction in weight of 1.6 per cent. Their paired controls gained 3.8 per cent in weight.

DISCUSSION

The most clearcut finding is that, contrary to general opinion for boys of this age, the obese group ate less than the non-obese in all cases. This is even more pronounced than in obese high-school girls of the same age, who ate significantly less than the non-obese but whose intakes did overlap to some degree with the lower part of the control range.[11] It was found that non-obese girls performed three times as much strenuous activity as did obese girls.

While it is true that in the present study there was no discernible difference between the obese and non-obese control groups in the amount of time recalled as spent in very active exercise during the school year, Dorris and Stunkard[3] observed that 7 out of 15 obese women grossly overestimated their own physical activity. It may well be that a similar situation prevailed as regards obese boys. At camp, every boy was encouraged to participate in a variety of activities, and in terms of total time spent, obese and non-obese did so equally as can be seen in part by the proportion of recorded time devoted to light, moderate, and very active exercise (Table VII). Certainly, the degree of enthusiasm for active sports shown during the time allotted to free-choice activities tended to be less for the obese boys than for their controls (Table VIII). This observation suggests that during the school year and the summer, the hours indicated as high expenditure activity periods might correspond to a lower rate of caloric expenditure for the obese than for the non-obese boys, even if body weight is taken into account.

The suggestion that obesity is associated with a decreasing amount of activity as weight increases[1] rather than with caloric intakes greater than average receives additional support from the finding that the five obese boys (mean age 14.1 yr) who spent more time in active sports at camp than their controls had a mean weight of

136 lbs at the beginning of the camp season and consumed an average of 2,919 cal daily in the school year. In contrast, the eight obese boys (mean age 14.3 yr) who spent less time in very active exercise than their paired controls had a mean weight of 143 lbs, but also consumed 2,984 cal daily for the school year. Likewise, the total daily caloric intake at camp was equal for boys who were more active and less active than the controls.

It was not surprising to find that some obese boys were more conscious of their food intakes at camp, and this may be reflected in part by the fact that from home to camp, the daily intakes of the obese group increased 472 ± 219 cal/day in comparison with the increase of 1,152 ± 367 cal found in the control group. Previous studies on the reliability of the method of assessment of dietary intake used here when applied to obese subjects[20,21] and the fact that observed food servings consumed checked as well for the obese as for the non-obese boys with reported intakes suggest that intakes presented here are close to the actual values. In accordance with the calorie allowances stated by the Committee on Calorie Requirements of the FAO, the recommended requirement for boys 13 to 15 years of age is 3,100 cal.[22] The obese boys in this study, on the average, were not consuming amounts of food above the expected requirement for this age group (Table IV).

CONCLUSION

The results presented indicate that obese boys, when compared with non-obese controls of the same age, height, and sexual maturation, ate significantly less than the non-obese boys both during the school year and at camp during the summer. Overeating, if understood in terms of eating more than average, was *not* typical of the obese boys. It is likely that they "over ate" only in a relative sense in that their energy expenditure was depressed below an energy intake which was moderate for their sex and age group.

SUMMARY

Comparison was made of the food eaten and the amount and degree of participation in physical activity of 14 obese adolescent boys in relation to that of paired control non-obese boys during a summer camp season and for the previous school year.

The energy intake of the obese boys was significantly less than that of the non-obese controls. Little difference was noted in the amount of time scheduled for light, moderate, and very active exercise but the degree of participation in the active exercises was observed to be generally less for the obese than for the non-obese

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Dietary Fat and Hypercholesteremia in the Cebus Monkey

III. Serum Polyunsaturated Fatty Acids

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THE Cebus monkey has been used extensively in this laboratory for studies of experimental atherosclerosis since it was first observed that this animal would readily accept a purified diet and would develop hypercholesteremia and atherosclerosis on certain diets.[1] The biochemical and morphologic responses of these monkeys to hypercholesteremic regimens seem to resemble those in man and to be different from those seen in certain lower animals in several respects, including the following: Marked elevations of serum cholesterol and beta-lipoprotein levels can be maintained for very long periods without histologic or chemical evidence of abnormal lipid accumulation in the liver or other organs;[1,2] the disappearance of orally administered radiocholesterol describes a two-phase exponential curve[2] with half-lives of magnitudes similar to those seen by Hellman *et al.*[3] in man; the ultracentrifugal and electrophoretic patterns of lipoproteins approximate much more closely those seen in man than do the patterns of rats;[4] and the vascular pathology of the Cebus monkey (compared to that of the rat), more closely simulates that of man, particularly with respect to the occurrence of spontaneous lesions, to the distribution of induced lesions and to certain histologic aspects.[31]

Cebus monkeys fed cholesterol[5] resemble both man[e.g.6,7,8] and several other species[9,10] in their response to different dietary fats with different serum cholesterol levels. Such differences are present regardless of the type of protein or carbohydrate fed. There is, of course, still considerable disagreement among investigators in this field about the factors in fats which influence serum cholesterol levels. The majority of workers,[e.g.11,12,13] perhaps, believe that part, if not all, of the serum cholesterol-regulating activity in natural fats is related to the nature of the fatty acids which are included in their component glycerides. The fats which are relatively rich in polyunsaturated fatty acids are associated with lower serum cholesterol levels than those fats which are less rich in polyunsaturated acids.

It seems clear that a great deal more information about the metabolism of polyunsaturated fatty acids will be required before crucial significance in regulation of cholesterol metabolism can be assigned to them or before their mechanism of action in this respect can be established. It has been shown that several hypercholesteremic stimuli[14] accelerate the time of appearance of essential fatty acid-deficiency signs in rats. Other evidence for a relationship between the essential fatty acid-deficiency syndrome in animals and cholesterol metabolism is less direct.

One approach to an understanding of the relationship of dietary polyunsaturated fatty

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acids and cholesterol metabolism has been the measurement of polyunsaturated fatty acid concentrations in tissues of subjects under various experimental conditions. The availability of methods for the separation of tissue lipids into various biochemical subclasses[15,16,17] before measurement of polyunsaturated fatty acids has further strengthened this approach.

This study describes the changes in polyunsaturated fatty acid concentrations in the serum lipids and in subfractions of serum lipids from Cebus monkeys which were fed diets containing different fats and in which cholesterol was added to the diet in certain experiments.

EXPERIMENTAL

Sixteen Cebus monkeys weighing between 1,500 and 2,100 g were used in these experiments. They were housed and fed as previously described.[1,5] All animals were offered 400 calories of diet including 17 g of protein (vitamin-free casein) daily. Cholesterol, when included in the diet, was supplied at 0.1 g per 100 calories of diet. The quantities of minerals and vitamins supplied were the same as previously reported.

Analytic Measurements

All sera were recovered from blood freshly drawn from Cebus monkeys just prior to offering the daily ration. The lipid determinations on sera were carried out by a procedure which was an adaptation of several published methods. Essentially, this consisted of the separation of serum lipids into three fractions: (a) cholesterol esters; (b) free cholesterol, triglycerides, and unesterified fatty acids; and (c) phospholipids on a silicic acid column of about 4 ml retention volume.* All fractions, including unseparated lipid extracts of sera, were hydrolyzed, cholesterol determinations were performed on ligroine extracts from the alkaline hydrolysate by the method of Abell *et al.*,[18] and polyunsaturated fatty acids were determined on ligroine extracts from the acidified hydrolysates. The linoleic lipoxidase method of MacGee and Mattson[19] (in which all polyunsaturated fatty acids with the *cis* configuration have essentially identical molar extinction coefficients) was used for all samples; in some cases the alkaline isomerization method of Holman and Hayes[20] was also applied to samples. All determinations were carried out in duplicate or triplicate.

RESULTS

Effect of Feeding Diets Containing Corn Oil or Coconut Oil on the Polyunsaturated Fatty Acid Content of Serum Lipids

Four monkeys were used in the first experiment. Prior to the experiment they were fed a basal purified diet, which supplied approximately 15 per cent of calories as corn oil and was essentially free of cholesterol. Two monkeys were then placed on a diet supplying 45 per cent of calories as corn oil and containing 0.1 g cholesterol per 100 calories of diet, and two were fed a similar diet with coconut oil as the sole source of fat. After 15 weeks on these diets the groups were reversed, the monkeys fed corn oil being changed to coconut oil diets and vice versa. At the end of 30 experimental weeks cholesterol was removed from the diets.

* The following materials have been found in the indicated fractions after separation on the chromatographic columns: Fraction *a*—cholesteryl laurate, cholesteryl linoleate (synthesized by a modification of the method of Page and Rudy,[30] using oxalyl chloride in lieu of thionyl chloride), cholesteryl palmitate, and cholesteryl stearate; Fraction *b*—cholesteryl acetate, unesterified cholesterol, triolein, oleic acid, linoleic acid, linolenic acid, and the major components of corn oil and linseed oil; and Fraction *c*—the lipid phosphorus of human and Cebus monkey sera. Recoveries ranged from 89 to 98 per cent for the various components, and separations were efficient at quantities of three or more times the quantities usually encountered in biological samples (lipid extract of 0.2 ml of sera). The technical errors (Technical Error = $\sqrt{\Sigma\Delta^2/2k}$, where k = number of pairs of duplicates and Δ = differences between duplicates) of separation and measurement of polyunsaturated fatty acids on 20 duplicate extracts of sera, each evaluated in true duplicate on successive days, were 9 per cent for total polyunsaturated fatty acids, 17 per cent for Fraction *a* (cholesteryl esters) polyunsaturated fatty acids, 20 per cent for Fraction *b* (triglycerides and unesterified fatty acids), and 22 per cent for Fraction *c* (phospholipids). The average differences from the mean of these 4 determinations were 5, 7, 11, and 9 per cent, respectively. The recovery of total polyunsaturated fatty acids in the three fractions of sera averaged about 91 per cent.

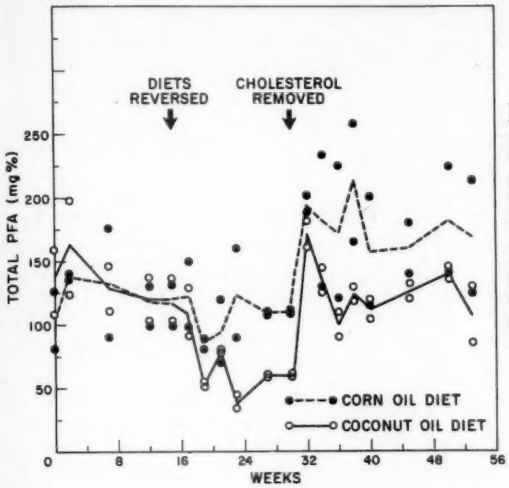

Fig. 1. Effect of diet on the concentration of total polyunsaturated fatty acids (PFA) in the sera of Cebus monkeys. The zero point on the time axis represents the steady state values when monkeys were fed the control diet which contained 15 per cent of calories as corn oil and no added cholesterol. At zero time 2 monkeys were given diets containing 45 per cent of calories as corn oil, and two were given diets with 45 per cent of calories as coconut oil. Cholesterol was supplied at 0.1 g per 100 calories of diet. At the end of 15 weeks the diet groups were reversed, and at the end of 30 weeks cholesterol was removed from the diet.

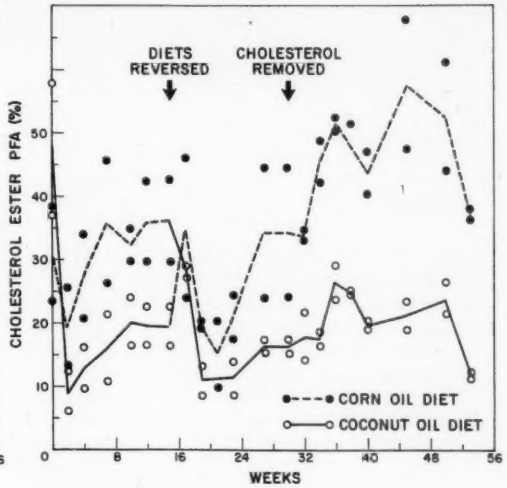

Fig. 2. Effect of diet on the concentration of cholesterol ester polyunsaturated fatty acids in the sera of Cebus monkeys. Values are expressed as percentages of the total fatty acids (molar basis) in the cholesterol ester fraction. See text and Figure 1 for dietary treatment.

Figure 1 is a representation of the total polyunsaturated fatty acid content of sera obtained from four monkeys fed corn oil or coconut oil diets at 45 per cent of calories. It can be seen that there was a tendency for the total polyunsaturated fatty acid content to decrease with progressive feeding of diets containing cholesterol. There was no difference between the values obtained for the two monkeys fed corn oil and the two fed coconut oil during the original 15-week period. After the reversal of diets, during the fifteen to thirtieth experimental weeks, the monkeys fed corn oil had higher total polyunsaturated fatty acid levels. After removal of cholesterol from the diet, the total polyunsaturated fatty acid levels in the sera of all monkeys rose dramatically. The depression of polyunsaturated fatty acid levels during the initial 30 experimental weeks was reflected in the concentrations of all chromatographic fractions.

The level of polyunsaturated fatty acids in the cholesterol ester fractions of the sera from these same four monkeys is indicated in Figure 2. The polyunsaturated fatty acid level is expressed as the percentage of the total fatty acids (on a molar basis) in the fraction. When the cholesterol-containing diets were fed during the first 30 experimental weeks, there was somewhat less than a twofold difference in the polyunsaturated fatty acid composition of the cholesterol ester fraction in sera of the coconut oil and corn oil groups. The mean figure for the corn oil group was about 28 per cent and 16 per cent for the coconut oil group. After removal of cholesterol from the diet the mean percentages of polyunsaturated fatty acids in the sterol ester fractions rose to 43 per cent and 19 per cent, respectively.

Effect of Feeding Fat-deficient Diets on Polyunsaturated Fatty Acid Content of Sera

Five Cebus monkeys were placed on essentially fat-free diets. The only significant source of lipid in the diet was approximately

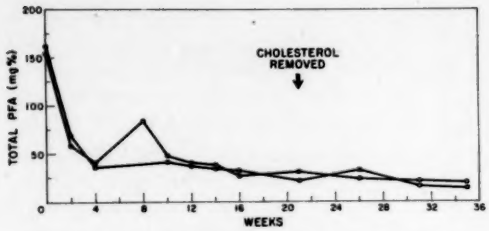

Fig. 3. Effect of feeding essentially fat-free diets on the total polyunsaturated fatty acids in the sera of Cebus monkeys. The zero time value represents the steady state determination when monkeys were fed the control diet containing 15 per cent of calories as corn oil and no added cholesterol. At time zero, 5 monkeys (2 represented here) were placed on fat-free diets which contained 0.1 g cholesterol per 100 calories of diet. At the end of 21 weeks, cholesterol was removed from the diet.

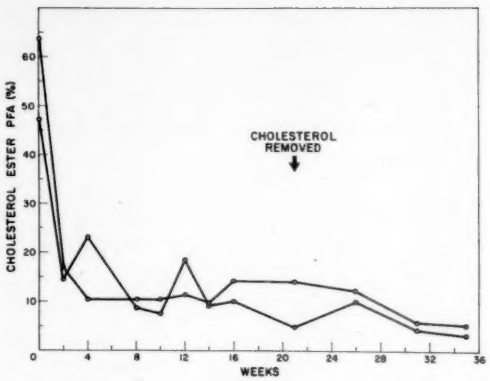

Fig. 4. Effect of feeding essentially fat-free diets on the concentration of cholesterol ester polyunsaturated fatty acids in the sera of Cebus monkeys. Values are expressed as percentages of the total fatty acids (molar basis) in the cholesterol ester fraction. See text and Figure 3 for dietary treatment.

35 mg of oleum percomorphum* in the daily ration. The monkeys readily accepted this diet and maintained body weight almost constant throughout the 35-week period of study. The only changes observed in the condition of the animals were the appearance of a dry skin which was disposed to cracking and visible desquamation (most prominent on the tail) and a change in the color of the hair. The hair of the monkeys which was initially dark brown on the dorsal surface of the body and tan on the ventral surface was changed so that the ventral surface was a bright yellow to orange.

Figure 3 shows the total polyunsaturated fatty acid concentration in the serum lipids of two representative monkeys. Time zero represents the steady state values on a diet containing corn oil and no cholesterol. The monkeys were placed on the fat-free diet with added cholesterol (0.1 g of cholesterol per 100 calories of diet), and at the twenty-first experimental week cholesterol was removed from the diet. The total polyunsaturated fatty acid level fell precipitously during the first two weeks and then declined more slowly.

Figure 4 shows the values for polyunsaturated fatty acids in the cholesterol ester fractions (expressed as percentages of the total fatty acids in the sterol ester fraction). Even after 35 weeks on the experimental diets, which were extremely low in lipid, the polyunsaturated fatty acid level in the sterol ester fraction was about 5 per cent. The serum cholesterol values (about 145 mg per 100 ml) for the cholesterol-containing diet were not significantly different from those for the cholesterol-free diet. The normal percentage of serum cholesterol in the esterified state (70 to 80 per cent) was maintained by monkeys on the fat-free diet.

Comparison of Mean "Steady State" Levels of Polyunsaturated Fatty Acids with Diets Containing Several Kinds and Levels of Fat

In Table I are shown mean values of serum polyunsaturated fatty acids for monkeys fed several different diets. Determinations were made after the monkeys had been on the indicated diets for two or more weeks. The total polyunsaturated fatty acid levels were influenced by the type of fat and by the presence of dietary cholesterol. The decrease of these levels associated with the feeding of cholesterol was accounted for by a decrease in the polyunsaturated fatty acids of all chromatographic fractions. The percentage of polyunsaturated fatty acids in the sterol ester fraction reflects somewhat the com-

* Concentrate of fat-soluble vitamins, Mead Johnson & Company, Evansville, Indiana.

TABLE I

Long-Term Effect of Feeding Different Fats on the Polyunsaturated Fatty Acid Composition of Sera from Cebus Monkeys

Diet*	No. determinations	Total PFA† (mg/100 ml)	Sterol ester PFA†	
			mg/100 ml	% of ester fatty acid
15 Corn	16	120.0	35.4	43.3
45 Corn	16	181.5	51.6	45.4
45 Corn Δ	20	115.1	35.6	27.6
45 Coconut	16	127.0	26.5	19.0
45 Coconut Δ	20	94.8	25.5	16.2
10 Safflower Δ	17	121.5	35.3	37.4
45 Safflower Δ	20	130.1	39.5	30.3
Fat-free	20	41.6	8.7	11.5‡

The values indicated are a composite of measurements made on monkeys which had been fed the indicated diets for periods of from 2 to 58 weeks.

* The figures under *Diet* indicate the percentage of calories derived from fat. Δ indicates the presence of cholesterol in the diet at 0.1 g per 100 calories.

† Polyunsaturated fatty acids.

‡ After 35 weeks on fat-free diets, the mean total PFA value was about 20 mg per cent and the figure for sterol ester PFA had fallen to about 5 per cent of the sterol fatty acids.

position of the dietary fat, although the differences in serum sterol ester polyunsaturated fatty acids are less than the differences in the composition of the dietary fats ingested. The feeding of coconut oil (about 2 per cent of fatty acids as linoleic) or the feeding of the essentially fat-free diets never resulted in levels of sterol ester polyunsaturated fatty acids of less than from 5 to 10 per cent.

Effect of Diet on the Types of Polyunsaturated Fatty Acids in Sera

Although it has been demonstrated that the total polyunsaturated fatty acid level in serum as determined by the linoleic lipoxidase method agrees rather well with the level as determined by the alkaline isomerization method, it was of interest to consider the degree of polyunsaturation (as can be determined by the latter method) in the serum lipids of monkeys fed different diets. The quantities of dienoic, trienoic, and tetraenoic fatty acids in the total serum lipids of monkeys

TABLE II

Effect of Diet on the Distribution of Serum Polyunsaturated Fatty Acids Expressed as Dienoic, Trienoic, and Tetraenoic Fatty Acids

Diet*	No. determinations	Serum polyunsaturated fatty acids (mg/100 ml)			Diene/Triene
		Diene	Triene	Tetraene	
15 Corn	8	120.3	23.6	13.0	5.10
45 Corn	9	172.5	14.6	20.5	11.81
45 Coconut	9	81.7	28.6	31.7	2.86
Fat-free	7	16.2	20.9	12.0	0.77
10 Safflower	9	114.6	16.1	29.0	7.12

Pentaenoic and hexaenoic acids were very low in all sera and are not listed here.

* The figures shown under *Diet* indicate the percentage of calories derived from fat; the name indicates the type of dietary fat.

fed five different diets are indicated in Table II. The values for pentaenoic and hexaenoic acids are not listed. In all cases the values for these two very unsaturated fatty acids were low, and these determinations were, perhaps, less reliable. The relative percentages as well as the absolute quantities of polyunsaturated fatty acids as dienoic acids were highest in those monkeys receiving the highest levels of dietary linoleic acid. The relative percentage of trienoic acids was highest in the animals receiving the lowest levels of dietary linoleic acid. The greatest absolute variation occurred, however, in the levels of dienoic acids. The relatively high level of tetraenoic acids in the monkeys fed coconut oil diets was, perhaps, a result of the fact that a relatively more significant amount of the low level of polyunsaturated fatty acids in this diet was contained in the added oleum percomorphum, which is rich in the very highly unsaturated fatty acids (the daily dosage of this vitamin supplement assayed 1.7 mg diene, 1.3 mg triene, 2.0 mg tetraene, 4.3 mg pentaene, and 3.6 mg hexaene; Klenk[21] has reported the specific structure of many of these unsaturated fatty acids in fish oils). The ratio of diene to triene varied from a high of 11.8 for the 45 per cent corn oil group to 0.77 for the "fat free" monkeys. After 6 months on the "fat free" diet the diene to triene ratio averaged around 0.5.

DISCUSSION

Clearly, dietary factors influence the polyunsaturated fatty acid content of serum lipids in the Cebus monkey. Similar effects have been demonstrated in several experimental animals[e.g. 22,23,24] as well as in man.[25,26,27] The inclusion of cholesterol in the diet of monkeys resulted in a depression of the total polyunsaturated fatty acid content of the sera. Also, monkeys fed diets with higher concentrations of polyunsaturated fatty acids tended to have somewhat higher levels of polyunsaturated fatty acids in sera. The serum levels, however, were not nearly so different as the polyunsaturated fatty acid concentrations of the dietary fat. The relatively much smaller differences in serum lipid composition, even after the various dietary fats were fed for periods of one year, suggest that the serum polyunsaturated fatty acids are selectively maintained—perhaps at the expense of tissue polyunsaturated fatty acids, or that the polyunsaturated fatty acids are synthesized *de novo*. It is true that a disproportionately high quantity of the polyunsaturated fatty acids in the coconut oil or fat-free groups was present as trienoic acids, not of dietary origin, per se. The differences in the polyunsaturated fatty acid content of the dietary fats were, however, greater than those seen even in the serum sterol ester fractions (whether one considered all polyunsaturated fatty acids or only the dienoic acids).

It is difficult to explain the apparently high quantities of tetraenoic acids found in the sera of monkeys, particularly those fed coconut oil diets. The methods have been sufficiently well tested to assure that the finding was not an artifact. The unwise decision to use oleum percomorphum, which is rich in the very unsaturated fatty acids, as the source of fat-soluble vitamins can conceivably explain the high tetraenoic acid levels in the sera of these monkeys. The observation that the diene:triene ratio was very low when the diet was relatively low in linoleic acid is consistent with the almost universal reports concerning other experimental animals[22,24] and man.[28]

It is interesting that in the Cebus monkey coconut oil diets do not produce very much higher serum cholesterol levels than do corn oil diets. Coconut oil has been shown to have less hypercholesteremic activity than does lard or hydrogenated cottonseed oil in these animals. Polyunsaturated fatty acid levels in sera have not been measured in monkeys fed lard or commercial hydrogenated cottonseed oil diets, although both of these latter fats have a range of linoleic acid content higher than that of coconut oil. Thus, although the polyunsaturated fatty acid content of the sterol ester fraction in monkeys fed corn oil is considerably higher than that of the sterol ester fraction in monkeys fed coconut oil, the serum cholesterol is only about 25 per cent higher in the monkeys fed coconut oil. In those monkeys fed a fat-free diet with or without dietary cholesterol, the serum cholesterol level is low and the sterol ester PFA level is also very low. If the fatty acid composition of sterol esters is of importance in their turnover, as many have suspected,[e.g. 27, 29] it is likely that characteristics of those fatty acids other than polyunsaturation will also prove to be important.

SUMMARY

The effect of dietary factors on the concentration of polyunsaturated fatty acids in serum lipids and in subfractions of serum lipids was studied in the Cebus monkey. When cholesterol was included in the diet the polyunsaturated fatty acid content of sera was reduced. The level of dietary polyunsaturated fatty acids influenced the level of polyunsaturated fatty acids esterified with cholesterol in serum. When Cebus monkeys were placed on diets essentially free of dietary fat, they developed certain hair and skin changes. The polyunsaturated fatty acid levels in the total serum lipids and in the serum cholesterol ester fractions of these monkeys was markedly depressed. The dienoic acids were the principal group affected.

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The Hypercholesteremic and Atherogenic Properties of Various Purines and Pyrimidines

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THERE IS still no agreement on the relative importance of various dietary factors in the development of atherosclerosis. Until recently the nitrogenous components of the diet had received little attention in regard to this disease. However, studies emanating from this[1–4] and other[5–8] laboratories indicate that dietary protein is an important consideration toward the understanding of the role of diet on the regulation of cholesterol metabolism. Furthermore, the quality[1] as well as the level[4] of the protein is believed to influence the development of experimentally induced atherosclerosis.

Another group of nitrogenous compounds, the purines and pyrimidines, is considered in the present report. It was shown in a preliminary communication that several of these compounds favor hypercholesteremia as well as an increased amount of incipient atherosclerosis in the rat.[9] Uracil, in particular, was found to be a potent hypercholesteremic agent. These studies were all carried out in the rat, since atherosclerosis, including coronary artery lesions, can be readily induced in this animal. One of the procedures for inducing this disease is to feed diets containing cholesterol and cholic acid. When dietary thiouracil, a sulfur-containing pyrimidine, is included, the pathogenic process is, as expected, accelerated.[3] The basal atherogenic regimen used in the following experiments contains cholesterol and cholic acid but no thiouracil.

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EXPERIMENTAL

The animals used were 12-week-old male albino rats of the Charles River strain which had been maintained on a diet consisting only of Purina Laboratory Chow prior to the introduction of the experimental regimens. All animals were then fed ad libitum a mild atherogenic regimen containing 0.5 per cent cholic acid, 1.5 per cent cholesterol, 20 per cent fat in the form of a hydrogenated cottonseed oil, 20 per cent casein, 54 per cent sucrose, 4 per cent salts, 0.2 per cent choline chloride, and a vitamin mixture as previously described.[2] To this regimen each of the various nucleic acids, purines, or pyrimidines to be studied was added at the levels indicated below. Control animals were fed this diet for the same period of time. These trials were not all executed concurrently, and for this reason it should be noted that the values for control animals are not the same for Figs. 1–3. Detailed comparisons of some of these compounds will be the subject of discussions elsewhere.[10,11]

A total of 78 rats are considered in the present report. The animals were bled at the end of 2, 3, 5, and 10 weeks of dietary treatment for the analyses of the various serum lipid components, including the total cholesterol.[12] At the end of this 10-week period each group was sacrificed, and the hearts,

aortas, kidneys, thyroids, and the other major organs were removed for histologic examination. The heart and aorta were opened in situ, fixed in 10 per cent formalin, and then stained with saturated Sudan IV in 70 per cent ethanol. The degree of cardiovascular sudanophilia was measured in ocular grid units with the aid of a dissecting microscope equipped with an ocular grid.[13] One hundred of these units is equivalent to 3.41 sq mm of surface.

RESULTS

Dietary Ribonucleic Acid (RNA) and Desoxyribonucleic Acid (DNA)

Either 1.5 per cent RNA or 1.5 per cent DNA was added to the atherogenic regimen; a third group received both 0.75 per cent RNA and 0.75 per cent DNA.

It was found that RNA was a slightly more potent agent than DNA for enhancing hypercholesteremia during the first five weeks of the study. However, at the end of the 10-week trial both dietary nucleic acids showed terminal responses of cardiovascular sudanophilia and hypercholesteremia that were not significantly different (Fig. 1). Among the rats which received the same atherogenic diet but with both 0.75 per cent RNA and 0.75 per cent DNA, a less marked over-all hypercholesteremic response was noted during the first 5 weeks. Although these animals finally attained the same magnitude of terminal cholesterolemia, the amount of cardiovascular sudanophilia was significantly less than seen among those groups receiving either RNA or DNA alone ($p < 0.01$). This finding suggests that when RNA and DNA are combined in the diet a protective synergism against arterial lipid deposition may occur.

Another group of seven animals previously had been fed higher levels of RNA, initially at a 3.3 per cent level, and as this diet was poorly tolerated the level was reduced eventually to 1.65 per cent.[9] In these animals hypercholesteremia was further aggravated. For example, at the end of a similar 10-week period, although endocardial sudanophilia was within the same range as above (e.g., 11.0), the mean serum cholesterol response was 840 mg per 100 ml. However, these higher levels of RNA resulted in striking degenerative changes in the aorta and renal lesions similar to, but less severe than, those observed in the rats described below receiving adenine supplementation.

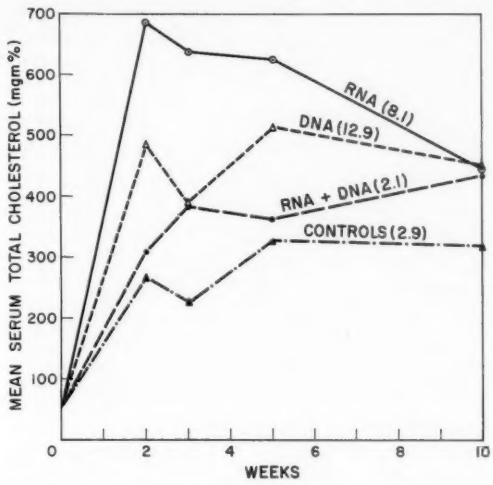

Fig. 1. Effect of dietary nucleic acids on hypercholesteremia and cardiovascular lipid deposition. The values in parentheses indicate the terminal amount of gross sudanophilia in the endocardium expressed in ocular grid units. Each point on the curves represents at least four and not more than twelve animals.

Dietary Purines

In this trial four purines are considered (Fig. 2). Uric acid, xanthine, and guanine were tolerated at a 3.3 per cent dietary level. These supplements were studied for 10 weeks. On the other hand, adenine was poorly tolerated and the dietary level was reduced to 1.65 per cent at the end of 3 days to 0.75 per cent at the end of one week, to 0.5 per cent at the end of two weeks, and finally to 0.33 per cent for the remaining 5 weeks of the study. This "adenine" group was finally sacrificed at the end of only 8 weeks because the survival of these animals was in doubt.

In each instance the addition of the purine to the basic atherogenic diet resulted in significant increments in the hypercholesteremia as well as moderate increases in endocardial

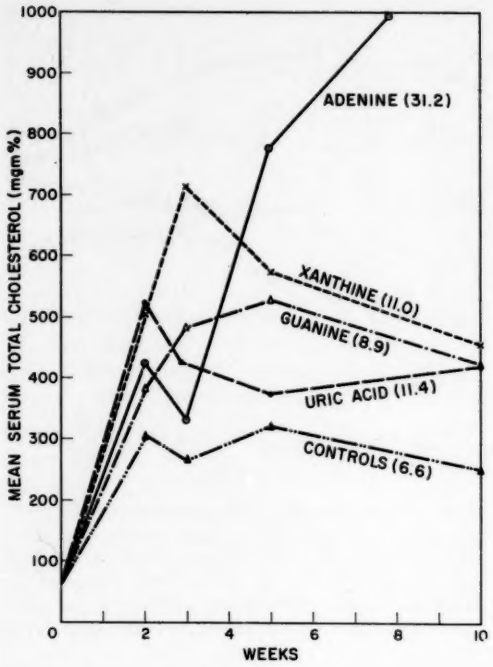

Fig. 2. Effect of various purines on hypercholesteremia and cardiovascular lipid deposition. The values in parentheses represent the amount of endocardial sudanophilia expressed in ocular grid units. Each point on the curve represents at least four and not more than eight animals, except among the rats treated with adenine where only three animals were available for terminal examination.

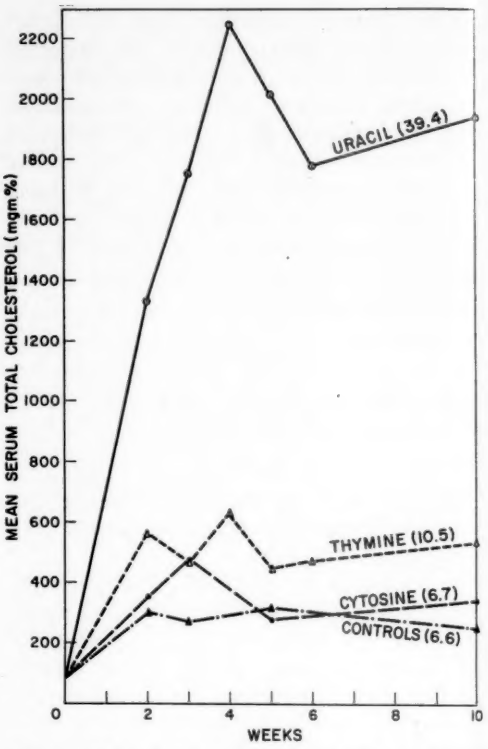

Fig. 3. Effect of various dietary pyrimidines on hypercholesteremia and cardiovascular lipid deposition. Each value in parentheses indicates the mean endocardial sudanophilia expressed in ocular grid units. Each point on the curve represents at least four and not more than eight animals.

sudanophilia. These changes were distinctly most pronounced among the group supplemented with adenine despite the lower dietary level (of purine) and the shorter duration of feeding. In addition, this group showed the same aortic and renal lesions previously seen in those animals receiving the massive levels of RNA,[9] though distinctly more advanced than in the latter. Similarly at a microscopic level both vascular and renal lesions from the two groups of animals were identical, the only differences being those of degree.

The aortic lesions consisted of patchy foci of degeneration in the media with loss of smooth muscle, collapse of elastic lamellae, and calcification. Overlying the larger lesions there was an apparently secondary proliferation of fibrous intimal plaques. Both the medial and intimal lesions were frequently though not invariably associated with moderate amounts of grossly visible sudanophilic lipid. These lesions were seen at all levels of the aorta, being most pronounced in the abdominal portion. Each of the major branches of the aorta was involved on occasion and microscopic involvement of coronary arteries, while not prominent, was present. The marked thinning of the media frequently resulted in aneurysmal dilatation, most pronounced in the ascending aorta. It should be stated that the aortic sudanophilia associated with these lesions was not included in the grading of endocardial sudanophilia. The renal lesions were characterized by the deposition within the cortical tubules of large amounts of

birefringent crystalline material. Acute inflammation associated with foreign-body giant-cell reaction and tubular dilatation were marked. To date these aortic and renal lesions have been seen only in those experimental groups receiving adenine or massive dietary doses of RNA. A more detailed report of the histologic changes will be reported elsewhere.[11]

Dietary Pyrimidines

Three pyrimidines were studied (Fig. 3). These pyrimidines were initially offered at a 3.3 per cent dietary level, but since this dose was poorly tolerated, as evidenced by mild anorexia, levels of 1.5 per cent were offered after the first week and continued during the remainder of the 10-week study. Uracil was the most potent of this group of compounds. The uracil-treated animals displayed an exceptional hypercholesteremia and extensive endocardial as well as aortic sudanophilia. In addition, the thyroid glands of these animals showed a histologic picture of hyperplasia similar to, though less marked than, that induced by thiouracil. No degenerative aortic changes or renal lesions such as were seen in the rats receiving dietary adenine were found in any of the groups supplemented with pyrimidines.

Animals treated with thymine had significantly less sudanophilia as well as less hypercholesteremia than those receiving uracil. Cytosine appeared to be the least reactive of this group.

DISCUSSION

It is interesting to note that both adenine, a purine, and uracil, a pyrimidine, potentiate vascular lipid deposition in the rat. It is not known at the present time exactly how each acts in this process. Although each is associated with an increased degree of hypercholesteremia, these effects seem to be mediated via different pathways. Uracil's action seems to be mediated via the thyroid gland, while adenine's action may be related to renal damage.

In another report a comparison of the hypercholesteremic, atherogenic, and thyroidal effects of uracil and thiouracil at various dietary doses will be presented.[10] To our knowledge, this uracil thyroid hyperplasia has not been heretofore appreciated, suggesting that thiouracil's hypothyroid action may not be entirely related to the presence of a sulfur moiety. With uracil, dietary levels of 0.25 per cent or higher are necessary to induce a thyroidal hyperplasia in acute studies such as described herein. Thus, it would appear that the efficacy of uracil in potentiating dietary hypercholesteremia may, in part at least, be mediated through the thyroid gland.

Of the various recent reports of experimental vascular lesions associated with induced renal damage, the studies of Lehr and coworkers would appear most pertinent to the present material.[14–16] These workers fed a highly insoluble sulfonamide to rats and rabbits which resulted in severe obstructive renal lesions. The latter lesions were associated with hyperfunction of the parathyroid glands and a resultant extensive medionecrosis of the aorta and its branches. Although certain differences were noted, there are marked similarities between the lesions observed by Lehr *et al.* and those herein reported. It is assumed that the lipid associated with the present mediodegenerative changes (and absent in Lehr's material) was induced by the dietary lipids and the resultant serum lipid abnormalities. In the animals described in our study renal damage was induced by feeding adenine with the deposition within the renal tubules of an insoluble adenine derivative, presumably 6,8-dioxyadenine. This lesion has been well defined.[17–19] It is of note that massive amounts of RNA (which contains adenine) produced renal lesions less severe than, but otherwise identical to, those produced by adenine.

At present, it is not clear to what extent the renal lesions may themselves play a role in the hypercholesteremic effect of adenine. There is little resemblance between the renal lesion induced with adenine and the nephrosis produced in rats by administration of amino nucleoside,[20–22] despite the presence in the configuration of the latter compound of a methylated adenine-like moiety. The amino nucleoside-induced lesion is accompanied by

a classical nephrotic syndrome including hypercholesteremia.

It is also interesting that when dietary RNA and DNA are added in equal amounts to an atherogenic diet, less early cardiovascular lipid deposition appears to take place than is seen with RNA or DNA alone, despite the fact that the animals receiving both RNA and DNA demonstrate a distinctly higher degree of hypercholesteremia than seen among the controls. This implies that, although the level of hypercholesteremia is related to the quantity of lipid deposit in vessels, processes may exist which can delay the deposition of excess lipid material in the intracellular and extracellular components of the tissue. This suggests either a more efficient lipid transport system or a more favorable metabolic system of catabolism and synthesis in the tissues to combat an exaggerated serum lipid load. Furthermore, these data substantiate the conclusion previously noted[4] that endocardial sudanophilia reflects serum cholesterol level to a reliable degree only within well-defined experimental parameters.

Thus, it appears that the atherogenic process can, perhaps, be severely affected by both the purines and pyrimidines. These present findings do not diminish the important position the lipids enjoy in the study of this disease, but serve to emphasize that in order to learn why arterial lesions develop one must be aware of all the metabolic disorders that accompany atherosclerosis. It is also interesting to note that certain patients with coronary artery disease also displayed abnormal serum uric acid levels.[23] Although the chief end product of purine metabolism in the rat is allantoin, as compared to uric acid in man, this species difference does not invalidate such comparisons; yet it certainly would be premature to relate the present experimental work directly to the human disease at this time.

SUMMARY

The hypercholesteremic and atherogenic properties of RNA, DNA, four purines, and three pyrimidines were assayed in rats fed moderately atherogenic diets for 10 weeks. It was found that either RNA or DNA supplementation alone favors a mild increase in hypercholesteremic response and cardiovascular sudanophilia. When RNA and DNA were combined in such diets, the level of cardiovascular sudanophilia was reduced to levels seen among the control rats.

Among the four purines studied, adenine was found to be the most atherogenic and hypercholesteremic. In addition, these animals displayed severe obstructive renal lesions and medionecrosis of the aorta and its branches, including the coronary arteries. The aortic lesions were associated with aneurysmal dilatation, fibrous intimal plaque formation, and lipid deposition. On the other hand, the rats fed either guanine, uric acid, or xanthine all demonstrated significant elevations in the serum cholesterol response and only mild increases in the amount of cardiovascular sudanophilia without the above renal damage or vascular necrosis.

Among the rats fed pyrimidines, uracil treatment resulted in a marked hypercholesteremia and cardiovascular sudanophilia. The nature of these changes as supported by thyroidal hyperplasia is reminiscent of the changes seen among rats treated with dietary thiouracil. This uracil effect on the thyroid therefore may account for the singular properties noted in this pyrimidine. The rats fed thymine demonstrated a mild increase in hypercholesteremia and cardiovascular sudanophilia, while the cytosine group's response was not significantly higher than the control rats. Significant thyroidal changes were not noted among these latter two pyrimidine groups.

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Effect of Pyridoxine Administration on the Urinary Excretion of Oxalic Acid, Pyridoxine, and Related Compounds in Mongoloids and Nonmongoloids

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THE RESULTS of two unrelated studies in this laboratory stimulated the present investigation. Gershoff *et al.*[1] have reported evidence suggesting the possibility of a methylating defect in mongoloids. In this same study a slight but statistically significant decrease in xanthurenic acid excretion following a tryptophan load test was observed in mongoloids as compared to nonmongoloid controls.

In another study Gershoff *et al.*[2] observed that in vitamin B_6-deficient cats large quantities of oxalic acid were excreted in the urine, which resulted in severe kidney damage. In the present work the effect of load tests of pyridoxine and folic acid in mongoloids and nonmongoloids has been studied.

EXPERIMENTAL

The subjects used in these experiments were either mongoloid or mentally deficient patients without other obviously abnormal characteristics. They ranged in age from 15 to 23 years. All of them lived in the same dormitory at the Wrentham State School and ate in the same dining room. Urine collections were made between 9:00 P.M. and 6:00 A.M. Vitamin loads were administered orally just prior to bedtime at 9:00 P.M. During collection periods the subjects were under continuous supervision to assure complete collections of urine. In the first study a set of control urines was obtained and the next night urines were collected after the administration of 10 mg pyridoxine hydrochloride and 5 mg folic acid. In a second study conducted six months later, using many of the same subjects, two sets of control urines were obtained and a load test of 20 mg of pyridoxine hydrochloride was given on the third evening.

In the first study the urines were analyzed for creatinine,[3] pyridoxic acid,[4] free and total vitamin B_6,[5,6] oxalic acid,[7] folic acid,[8] and inorganic sulfate.[9] Analyses for pyridoxic acid and oxalic acid were done on the urines collected in the second study.

RESULTS

The results of these experiments are summarized in Table I. In both experiments urinary oxalate decreased following the administration of the vitamin loads. The mean values obtained in the mongoloids and nonmongoloids were not significantly different. Following the administration of 10 mg of pyridoxine and 5 mg of folic acid, all 24 subjects showed a decreased oxalate excretion which averaged 58 per cent and was statistically significant ($p < .001$) by the t test. In the second study the feeding of 20 mg of pyridoxine was followed by

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TABLE I

The Effect of Pyridoxine and Folic Acid on Urinary Metabolites of Mongoloids and Nonmongoloids

	Mongoloids		Nonmongoloids	
	Before load	After load	Before load	After load
	NO. SUBJECTS = 12		NO. SUBJECTS = 12	
Pyridoxine 10 mg *Folic acid* 5 mg				
Creatinine (mg)	514 ± 65	451 ± 39	438 ± 32	411 ± 36
Pyridoxic acid (mg)	0.91 ± .10	3.22 ± .21	1.23 ± .13	2.72 ± .21
B_6, total (μg)	95 ± 7	257 ± 27	88 ± 9	381 ± 43
B_6, free (μg)	24 ± 3	91 ± 10	36 ± 5	145 ± 20
Inorganic sulfate S (mg)	258 ± 27	310 ± 31	291 ± 28	271 ± 28
Folic acid (μg)	3.0 ± .2	641 ± 83	3.1 ± .3	612 ± 68
Oxalic acid (mg)	12.4 ± 1.4	4.7 ± .6	12.1 ± .9	5.5 ± .8
	NO. SUBJECTS = 10		NO. SUBJECTS = 12	
Pyridoxine 20 mg				
Pyridoxic acid (mg)	1.26 ± .15	6.96 ± .42	1.19 ± .08	5.08 ± 1.02
Oxalic acid (mg)	11.7 ± .12	9.4 ± .8	11.8 ± .8	8.4 ± 1.3

All values include standard errors of the mean.
Vitamin B_6 values are expressed as pyridoxine hydrochloride.

decreased oxalate excretion in 18 of the 22 subjects. The average decrease of the entire group was 25 per cent and was statistically significant ($p < .005$).

Differences in the excretion of vitamin B_6 and its metabolites by mongoloids and nonmongoloids following the load tests were observed. The differences in the pyridoxic acid and vitamin B_6 values in the urines before and after the load tests gives an estimate of the recovery of the vitamin. In the first test nonmongoloids excreted 14.9 ± 2.0 per cent* and mongoloids 23.1 ± 1.2 per cent* of the administered 10 mg of vitamin B_6 as pyridoxic acid in 9 hours. These values are significantly different ($p < .01$).

In the second study nonmongoloids excreted 19.5 ± 5.2 per cent and mongoloids 28.6 ± 2.0 per cent of the 20 mg vitamin B_6 load as pyridoxic acid. The data obtained on the nonmongoloids included one excessively high value of 66.1 per cent. If this value was not included in the calculations the recovery of vitamin B_6 as pyridoxic acid in the nonmongoloids would have been 15.2 ± 3.3 per cent and the differences in the recovery values would again yield a p value of less than .01.

The urinary excretion of free and total vitamin B_6 unlike pyridoxic acid was greater in the nonmongoloids than mongoloids. Differences in free and total vitamin B_6 excretion between control urines and urines obtained following the 10 mg pyridoxine load were 67 ± 10 and 162 ± 32 μg, respectively, for mongoloids and 109 ± 19 μg and 294 ± 40 μg for nonmongoloids.

There were no significant differences in urinary folic acid and inorganic sulfate excretions between mongoloids and nonmongoloids.

DISCUSSION

The most striking observations in these studies were the difference in the excretion of vitamin B_6 and pyridoxic acid between mongoloids and nonmongoloids and the marked and rapid effect of vitamin B_6 in decreasing urinary oxalate, most of which is generally thought to originate from exogenous food sources.

All of the subjects, both mongoloid and nonmongoloid, are presumed to have been receiving adequate dietary vitamin B_6 and presented none of the known symptoms of vitamin B_6 deficiency. Although determinations of vitamin B_6 metabolites in the urine are not considered adequate tests of vitamin B_6 nutrition,[10] they may be used to approximate roughly the level of dietary pyridoxine. Linkswiler and Reynolds[11] found that in three subjects consuming 2.65 to 3.21 mg of vitamin B_6 per day an

* All values include standard errors of the mean.

average of 140 μg of vitamin B_6 and 3.54 mg of pyridoxic acid were excreted in the urine daily. Sarett[12] has reported that in his experiments and those of others, humans eating self-selected diets excreted an average of slightly more than 3 mg of urinary pyridoxic acid daily. Calculating the nine hour urinary excretion values of vitamin B_6 and pyridoxic acid observed in control urines in these experiments on a 24-hour basis gives comparable values. Thus, it is reasonable to assume that the subjects studied were receiving more than 1 to 2 mg of vitamin B_6, which is the amount suggested as the necessary daily intake by the Food and Nutrition Board.[13]

In vitro studies have shown that the activity of enzymes associated with sulfur metabolism is rapidly reduced in vitamin B_6 deficiency.[14] Gershoff *et al.*[2] have reported a decrease in urinary inorganic sulfate excretion in vitamin B_6-deficient cats. Assuming that similar relationships exist in humans, it appears from this study that the rate of excretion of oxalate is more sensitive to dietary vitamin B_6 levels than that of inorganic sulfate.

It is difficult to interpret the differences in the excretion of vitamin B_6 and pyridoxic acid in mongoloids and nonmongoloids following the load tests. These differences were not observed in control urines. It appears that mongoloids oxidize pyridoxine to pyridoxic acid more rapidly than do nonmongoloids. This may account for the greater excretion of vitamin B_6 in the urine of the nonmongoloids following the load tests. There were no significant differences between groups in the excretion of folic acid before and after the load test. Other studies will be necessary to explain the difference in methylation previously reported.[1]

This is the first study to relate oxalate excretion in humans to dietary vitamin B_6. In view of the role of oxalic acid in a number of human diseases,[2] it is of considerable interest to observe the marked decrease in urinary oxalate obtained in humans receiving an apparently adequate amount of vitamin B_6 when load tests of vitamin B_6 were given. The evaluation of urinary oxalate excretion as a functional test for adequacy of vitamin B_6 and the value of vitamin B_6 in the treatment of diseases associated with oxalate deposition or oxaluria, particularly oxalosis and various types of kidney disease, require further investigation.

SUMMARY

The excretion of various metabolites by mongoloid and nonmongoloid mentally deficient patients has been studied prior to and following the oral administration of pyridoxine and folic acid. The excretion of oxalic acid by both groups was markedly reduced by pyridoxine administration although the subjects had been receiving a diet apparently adequate in vitamin B_6. Following pyridoxine administration, mongoloids excreted more pyridoxic acid and less vitamin B_6 than did nonmongoloids. No significant differences in folic acid excretion were observed in the two groups studied.

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Hypoglycemic Effects of Saccharin in Experimental Animals

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SACCHARIN (o-sulfobenzoic acid imide) is commonly used as a physiologically inert sweetening agent and, as such, is extensively used by diabetics as a substitute for sucrose in the diet. The British Artificial Sweeteners in Food Order (1953) states that saccharin is known to be harmless, while the use of other sweeteners may be detrimental to health. Studies of the toxic effect of saccharin in animals has been reported by Fitzhugh *et al.*,[1] Fantus and Hektoen,[2] and Carlson and associates,[3] and in many by Herter and Folin.[4] In these studies no attempt was made to determine the effect of saccharin on the blood sugar. Several other early reports describe a decrease in the blood sugar following administration of saccharin,[5–8] several report on significant change,[9–11] while Syllaba[12] observed an increase in rabbits and in man following administration by stomach tube. The majority of these experiments were performed on fasted normal human subjects. Unfortunately, the earlier reports show not only a paucity of experimental detail but also a lack of statistical inference.

It therefore appeared useful to determine the effect of both chronic and acute administration of saccharin on the blood sugar levels of both fed and fasted rats and mice. The mode of administration was varied to determine whether the effect was independent of the mode. A second report will describe the effect of saccharin in human subjects.

In view of the report by Macallum and Sivertz[13] that sulfonamides, including saccharin, potentiate and accelerate the hypoglycemic action of insulin when injected concomitantly, it seemed of especial interest to observe the effect on the obese-hyperglycemic mice (Bar Harbor *obob* strain) being studied in this laboratory. These animals present among other metabolic characteristics an insulin-resistant hyperglycemia, an increased pancreatic content of both insulin and glucagon,[16] and increased circulating insulin.[17]

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MATERIALS AND METHODS

Three types of animals were used in these experiments: obese-hyperglycemic mice of both sexes (40 to 70 g, averaging 57 g), their lean littermates (24 to 40 g, averaging 30 g), and female Wistar adult rats (250 to 300 g). All animals were kept in separate cages at constant environmental temperature (78°F) and under regular illumination. The effect of both chronic and acute administration was studied.

For the blood sugar determination, 0.1 ml of whole blood was obtained from the tail vein; assay was performed using the method of Somogyi,[14] with the colorimetric reagent of Nelson.[15] Blood was taken from animals under mild Nembutal anesthesia (10 mg/Nembutal/100 g mouse and 5 mg/rat).

I. Chronic Experiment

Two diets were used in the chronic experiment, a "synthetic" high-carbohydrate diet and ground Purina lab chow. The "synthetic" diet was of the following composition: casein 25%, corn oil 3%, cod liver oil 2%, sucrose 65.7%, Hegsted salt mix 4%, cystine 0.2%, and choline chloride 0.1%. The diet was supplemented with the following vitamins per kilogram diet: thiamine 10 mg, pyridoxine 10 mg, riboflavin 20 mg, niacin 50 mg, pantothenic acid 100 mg, biotin 0.2 mg, and folic

TABLE I

Food Consumption (g) of Obese-Hyperglycemic Mice and Their Lean Littermates on a Ground Purina Lab Chow plus 25 g Saccharin/kg Diet

Week	Obese			Lean		
	Control (N = 10)	Test (N = 10)	p	Control (N = 10)	Test (N = 10)	p
1	37 ± 4	36 ± 8	—	29 ± 9	26 ± 6	—
2	40 ± 7	40 ± 7	—	36 ± 6	34 ± 4	—
3	49 ± 17	62 ± 9	<0.05	35 ± 6	35 ± 6	—
4	38 ± 8	50 ± 7	<0.05	35 ± 5	39 ± 6	<0.05

TABLE II

Blood Glucose Levels (mg per 100 ml) of Obese Hyperglycemic Mice and Their Lean Littermates in Chronic Experiment: High-Carbohydrate Diet with Saccharin Added in Amounts Indicated

Day of experiment	Saccharin (g/kg diet)	Obese			Lean		
		Control (N = 10)	Test (N = 10)	p	Control (N = 10)	Test (N = 10)	p
t_0	—	149 ± 21	165 ± 5	—	81 ± 19	82 ± 19	—
t_7	5	153 ± 20	148 ± 22	—	76 ± 8	76 ± 11	—
t_{14}	5	178 ± 22	169 ± 22	—	89 ± 13	89 ± 10	—
t_{18}	25	171 ± 16	62 ± 22	<0.01	81 ± 16	81 ± 10	—
t_{25}	25	149 ± 11	59 ± 30	<0.01	96 ± 15	91 ± 12	<0.02

acid 5 mg. Vitamins A and D were present in the commercial Norwegian cod liver oil in the potency of 1800 and 180 units per gram, respectively.

Twenty obese and twenty lean mice were used in each of the chronic experiments; each group was further subdivided into test and control animals and received the appropriate diet. The "control" diet contained no additions, while saccharin* in the amounts indicated was added to the "test" diet. As nearly as possible equal numbers of male and female mice were tested. Twenty test and twenty control rats were used, all individually caged. All animals were allowed to eat ad libitum during both experiments. Blood was taken from animals in the fed state.

Each experiment was run for the period of four weeks. Blood sugar was determined on each individual animal prior to and at designated intervals during the experiment. Weight (once a week) and food intakes (every other day, corrected for spilling) were determined routinely throughout the experiment.

* Saccharin Sodium (Soluble Powder U.S.P., Lot No. B96), supplied by Monsanto Chemical Company, St. Louis, Mo., was used throughout the experiment.

II. Acute Experiment

In the acute experiment both the mode of administration and the physiologic status of the animals was varied. In contrast to the chronic experiment both fed and fasted animals were used. Again obese and lean mice were tested, in addition to female Wistar rats. Administration of the saccharin solution was made both by intraperitoneal injection and by stomach tube. Each test rat received 100 mg saccharin, while each test mouse received 10 mg, and their control counterparts received an equal volume of distilled water and similar handling. Rats were fasted for 24 hours and mice for 15 hours. The 24-hour food intakes were determined immediately preceding and after the administration. Blood samples were taken prior to and 30 minutes after the administration. The blood sugar determination was identical to that of the chronic experiment.

RESULTS

I. Chronic Experiment

A. *Food Intake.* During the first experiment ("synthetic" high-carbohydrate diet), the test animals consumed neither more nor less than their controls—the rats 11 to 14 g,

TABLE III

Blood Glucose Levels (mg per 100 ml) of Obese-Hyperglycemic Mice and Their Lean Littermates in Chronic Experiment: Ground Purina Lab Chow with Saccharin Added

Day of experiment	Saccharin (g/kg diet)	Obese			Lean		
		Control (N = 10)	Test (N = 10)	p	Control (N = 10)	Test (N = 10)	p
t_0	—	164 ± 15	152 ± 18	<0.05	85 ± 17	87 ± 7	<0.05
t_7	25	149 ± 36	128 ± 16	<0.05	95 ± 18	77 ± 14	<0.05
t_{14}	25	161 ± 30	98 ± 5	<0.01	110 ± 14	101 ± 14	<0.05
t_{21}	25	155 ± 28	95 ± 46	<0.01	106 ± 22	73 ± 15	<0.01
t_{28}	25	166 ± 54	*82 ± 34	<0.01	98 ± 13	78 ± 15	<0.05

* Two test mice died.

TABLE IV

Weight of Female Adult Wistar Rats (grams) in Chronic Experiment: High Carbohydrate Diet with Saccharin Added in Amounts Indicated

Day of experiment	Saccharin (g/kg diet)	Test (N = 12)		Control (N = 12)		
		Weight	% Change	Weight	% Change	p
t_0	—	250 ± 16	0	252 ± 18	0	>0.05
t_7	5	246 ± 12	−1.6	261 ± 22	+3.6	>0.05
t_{14}	5	248 ± 12	−0.8	264 ± 23	+4.8	>0.05
t_4	25	249 ± 11	−0.4	276 ± 25	+9.5	<0.01
t_{25}	25	249 ± 15	−0.4	276 ± 28	+9.5	<0.01

the obese-hyperglycemic mice 5 to 7 g, and the lean mice 3 to 5 g daily. However, when ground Purina chow was the dietary medium, the obese test mice consumed significantly more than their controls; a less marked increase was noted in the case of the lean mice (Table I).

B. *Blood Sugar Levels.* The blood sugar values for the obese and the lean mice receiving the high-carbohydrate diet are shown in Table II according to concentration of saccharin in the test diet. Saccharin had a hypoglycemic effect in the obese-hyperglycemic mice only, and only in the concentration of 25 g/kg diet.

Saccharin in the concentrations used had no appreciable effect on the blood sugar levels of normal rats. In the high-carbohydrate experiment, saccharin up to 25 g/kg diet was added; the blood sugar of both test and control animals were in the range of 80 ± 3 mg per 100 ml glucose ($p > 0.05$).

A hypoglycemic effect was also seen when ground Purina chow is the basal diet (Table III). Again the decrease in the obese mice was of greater magnitude than that in the lean littermates; the percentage decrease was also greater.

C. *Weight.* There did not appear to be a weight loss in either of the two types of mice when maintained on the test diet (Table IV). There was a weight decrease in the test rats, however, which was especially marked when the animals were on a diet containing 2.5% saccharin ($p < 0.01$). This observation supports that of Fitzhugh *et al.*[1] that rats on 5% saccharin were smaller than control ($p < 0.05$).

In an attempt to relate this data to previous reports, the amount of saccharin ingested daily by an "average" animal in each of the three

TABLE V

Calculated Amount of Saccharin (mg) Consumed Daily by the Three Types of Animals: Chronic Experiment with Two Dietary Media

Species	High carbohydrate diet			Ground Purina chow	
	Daily consumption (g)	Saccharin 0.5%	Saccharin 2.5%	Daily consumption (g)	Saccharin 2.5%
Obese mice	5–7	25–35	125–175	6–9	150–225
Lean mice	3–5	15–25	75–125	4–5	100–125
Rats	11–14	55–70	275–650	—	—

TABLE VI

Blood Glucose Levels of Obese-Hyperglycemic Animals, Their Lean Littermates, and Normal Female Adult Wistar Rats in Chronic Experiment: Ground Purina Lab Chow; Administration of Saccharin Solution by Method Indicated; Tail Blood Taken Before and 30 Minutes After Administration

Animal		No.	Weight (g)	mg % glucose			
				t_0	t_{30}	Diff.	p
Fasted, intraperitoneal saccharin							
Obese	Control	9	58 ± 6	95 ± 23	91 ± 19	− 4	>0.05
	Test	9	51 ± 5	103 ± 43	98 ± 38	− 5	>0.05
Lean	Control	10	26 ± 3	74 ± 14	84 ± 10	+10	>0.05
	Test	10	25 ± 3	84 ± 18	75 ± 20	− 9	>0.05
Rats	Control	6	284 ± 15	74 ± 16	65 ± 13	− 9	>0.05
	Test	6	236 ± 19	62 ± 6	59 ± 5	− 3	>0.05
Fed, intraperitoneal saccharin							
Obese	Control	15	48 ± 7	191 ± 28	180 ± 13	−11	>0.02
	Test	14	54 ± 5	184 ± 19	113 ± 32	−71	<0.01
Lean	Control	11	26 ± 5	108 ± 10	109 ± 10	+ 1	>0.05
	Test	12	23 ± 4	107 ± 18	68 ± 20	−41	<0.01
Rats	Control	18	276 ± 26	85 ± 9	83 ± 10	− 2	>0.05
	Test	18	285 ± 30	85 ± 8	70 ± 14	−15	<0.01
Fed, saccharin by stomach tube							
Obese	Control	10	52 ± 5	160 ± 25	169 ± 22	+ 9	<0.05
	Test	10	55 ± 5	175 ± 25	183 ± 33	+ 8	<0.05
Lean	Control	10	30 ± 9	105 ± 10	109 ± 21	+ 4	>0.05
	Test	10	27 ± 4	114 ± 6	114 ± 16	0	>0.50
Rats	Control	12	271 ± 23	85 ± 7	78 ± 9	− 7	>0.05
	Test	11	278 ± 19	85 ± 13	87 ± 7	+ 2	>0.05

groups was calculated (Table V). It is apparent that the maximum doses administered chronically were well in excess of the 20 to 50 mg of saccharin indicated in many of the references cited.

II. Acute Experiment

Table VI contains a summary of the data obtained in the acute saccharin experiment. The method of administration of the saccharin and the physiologic status of the animals are defined for each group. It is evident from the data presented here that a hypoglycemic effect was not observed when the saccharin was administered by stomach tube to fed animals nor when animals were in the fasted state.

DISCUSSION

The data presented in this paper indicate that saccharin produces a hypoglycemic effect under certain defined conditions. The hypoglycemic effect is seen in fed normal rats given intraperitoneal injection of 100 mg saccharin, in obese hyperglycemic mice after chronic administration of 150 ± 25 mg saccharin per day, and in fed obese mice and their lean littermates after intraperitoneal injection of 10 mg saccharin.

Furthermore, under no conditions does the administration of saccharin cause an increase in blood sugar greater in magnitude than that observed in their controls, which received water and similar handling. In our opinion the hyperglycemic response reported by Syllaba[12] after administration by stomach tube (per os) may very possibly be due to the stress produced by the trauma of intubation. No controls were run in his experiment to demonstrate the effect of intubation per se.

To our knowledge there is no report in the literature on the effect of a chronic administration of saccharin on the blood sugar. The observation made in this paper—that in this situation hypoglycemic response is elicited in the obese-hyperglycemic mice only—is interesting when one considers the previously men-

tioned findings of Macallum and Sivertz[13] and the fact that the obese-hyperglycemic mice have been shown to have not only an increased pancreatic insulin but also a greater amount of circulating insulin than their non-obese controls.[18] Whether chronic administration would have produced a hypoglycemic effect in the lean mice if the duration of the experiment had been lengthened is not known at present.

It is difficult to explain this phenomenon. The action of saccharin may depend upon a property peculiar to saccharin, or it may act in a mechanism analogous to that of the group of oral hypoglycemic sulfonylureas being studied as possible "insulin substitutes." Several theories have been mentioned:

(1) Saccharin causes a reflex release of insulin mediated by the gustatory nerves. This theory has been proposed by Kun and Hormath[8] and by Jorgenson,[5] and is supported by their observation that the hypoglycemia is produced as readily after washing the mouth with saccharin as by drinking the saccharin solution. It is the "sweet taste" of saccharin which is responsible for its hypoglycemic effect, a property not common to the sulfonamides as a group. The effectiveness of saccharin after intraperitoneal injection would appear to refute this theory.

(2) Proposed mechanisms of the hypoglycemic effect of the sulfonylureas and sulfonamides in general: (a) they effect a release of insulin from the pancreas—i.e. "pancreatropic effect;"[18] (b) they reduce insulin degradation, inhibit "insulinase activity."[19] This theory has been refuted by Vaughan[20] and by Berson *et al.*;[21] (c) they may act as "alpha cell cytotoxins," destroying the cells which produce glucagon and thereby producing a relative excess of insulin;[22] (d) the hypoglycemic agents may reduce the response to epinephrine and/or glucagon or act directly on the phosphoglucomutase or phosphokinase system;[20] (e) there may be a "selective interference with hepatic gluconeogenesis;"[23] and (f) they inhibit glucose-6-phosphatase activity.[24,25]

Against the theory that a mode of action common to saccharin and to the commonly used oral hypoglycemic agents is the fact that it has been demonstrated in this laboratory that carbutamide is ineffective in reducing the blood sugar of the obese-hyperglycemic mice,[17] so that it appears more likely that saccharin may act by a selective and unique mechanism. An explanation of this action must also await further experiments.

SUMMARY

A study was made of both chronic and acute administration of saccharin to obese-hyperglycemic mice, their lean littermates, and adult female Wistar rats. Hypoglycemia was produced in obese-hyperglycemic mice following chronic administration of 125–175 mg saccharin daily; no change in blood sugar was seen in the other animals.

Acute intraperitoneal injection of 100 mg and 10 mg saccharin to fed rats and to the fed mice, respectively, was also effective in lowering the blood sugar. There was no reduction when the same doses were injected into fasted animals or when administration was by stomach tube.

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Triglyceride Utilization by Human HeLa and Conjunctiva Cells in Tissue Culture

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UNLIKE other major groups of nutrients such as carbohydrates, amino acids, and inorganic ions, lipids have thus far received relatively little attention in nutritional and biochemical studies employing cells maintained in continuous culture in vitro. It has been rather general procedure to avoid lipemic sera, often using fasted or at least preprandial donors. Toxic manifestations of unesterified fatty acids and their soaps are well known, and the surface activity of phosphatides, monoglycerides, and diglycerides has not favored their intentional inclusion in tissue culture media. In view of the fact, however, that most such media contain fresh or dialyzed serum, lipids at least in the form of lipoproteins are present. Recently, Cailleau *et al.*,[1] in a study of the reasons underlying the toxicity of certain sera, analyzed both "toxic" and nontoxic sera for their lipid concentrations and compositions. Their data revealed a higher lipid content for toxic sera and especially an elevation in free fatty acid content. Several investigators have included lipids in their media, especially where natural products such as serum have been omitted. A medium employed fairly extensively by Evans and associates[2] contained three unsaturated fatty acids: methyl linoleate, methyl linolenate, and methyl arachidonate. More recently, the three fatty acids have been omitted without noticeable effect.[3] Sato, Fisher, and Puck[4] have presented data showing the requirement for cholesterol by the S3 clonal strain of HeLa cells.

In the present studies, human HeLa and conjunctival cells have been incubated with a number of fat emulsions and similar preparations, and the effect on net cell multiplication has been determined. Emulsions containing glyceryl triolein-1-C^{14} and glyceryl tripalmitin-1-C^{14} have also been employed, and utilization of these triglycerides has been shown.

EXPERIMENTAL

The human cells used were of the HeLa and conjunctival (Chang) strains derived from carcinoma and normal conjunctiva, respectively.‡ Stocks of these cells were grown in Eagle's medium[5] containing 10 per cent horse serum and were transferred routinely about every eight days. The cells were checked at frequent intervals for possible contamination by pleuropneumonia-like organisms.§ Roller tube cultures were prepared from the stocks when approximately 3 to 5 $\times$ 10^6 cells were present, using the trypsinization technic previously described.[6] An inoculum of about 25 $\times$ 10^3 cells was used and the tubes were incubated on stationary racks at 35° C for 24 to 48 hours and were then rotated at a drum speed of 1 rpm at the same temperature. Eagle's medium was employed containing 10 per cent horse serum which had been dialyzed at 4° C. Additions of lipid preparations were made at times shown in the appropriate table. At the end of the experiments, cell counts were done as previously described.[7] Frequent micro-

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‡ Clonal strains of these cells were kindly furnished by Dr. R. S. Chang of the Department of Microbiology.

§ The authors are indebted to Dr. E. Murray of the Department of Microbiology for examining the slides in these tests.

TABLE I
Composition of Emulsion Preparations

Preparation No.	Component and Per Cent*					
	Coconut oil	Cottonseed oil	Corn oil	Pluronic-F68	Phosphatide	Ethyl alcohol
1	10	—	—	2.5	—	8
2, 3, 4	—	10	—	2.5	—	8
5	—	10	—	—	2	—
6	—	10	—	2.5	2	8
7	—	—	10	2.5	—	8
8	—	—	—	2.5	—	8
9†	—	15	—	0.3	1	—
10	—	—	—	—	—	8

* Per cent expressed as weight per volume. All preparations were made to final volume with 5% dextrose solution.

† Lipomul I.V., Upjohn Company.

scopic examinations were done throughout the experimental period.

The nonradioactive emulsions used were prepared by high-pressure homogenization and sterilized as has been described.[8] Except where otherwise noted, the composition was as follows:

	Weight/volume, %
Triglyceride	10.0
Pluronic-F68*	2.5
Ethyl alcohol†	7.0
Dextrose‡	4.7
Pyrogen-free H_2O§	to 100 ml.

* Furnished by Mr. Phelps Trix of the Wyandotte Chemical Corporation, Wyandotte, Michigan. This material is prepared by the condensation of ethylene oxide with polyoxypropylene, and has a molecular weight of approximately 8,000. In vivo tests have shown this material to be of extremely low toxicity.[9,10]

† Redistilled shortly before use.

‡ Merck and Company, Rahway, New Jersey.

§ Obtained from the Massachusetts General Hospital Pharmacy.

The emulsions have few fat particles greater than 1 μ and the average visible under the phase microscope is well below this. A large portion of the particles are beyond the resolving power of the light microscope.

The triglycerides used were a purified cottonseed oil,* coconut oil (Cobee 76)† and corn oil.‡ In addition to the Pluronic-stabilized emulsions, two phosphatide-stabilized emulsions were also tested. One of these was a commercial product§ and the other was a 10 per cent cottonseed oil emulsion prepared in this laboratory and contained 2 per cent of the soybean phosphatide¶ as the sole stabilizer. For control purposes, some preparations were prepared without fat. All materials were stored at 5° C. Media containing the various preparations were prepared every two days, and all media were changed every two days.

Radioactive emulsions were prepared by the following procedure: the radiolipide was dissolved with or without carrier triolein|| in 1 ml of redistilled acetone with warming. This solution was rapidly expelled from a small volume syringe through a #20 needle into a 1 ml volume of aqueous solution 2 per cent Pluronic-F68 and 5 per cent dextrose. Acetone from the resulting almost invisible emulsion was then removed by aeration with water pump N_2. The volume was adjusted to 1 ml. Sterile technic was employed throughout the procedure. The emulsions were used immediately after preparation. Radioactive triolein°

* Kindly supplied by Dr. Curtis Meyer of the Upjohn Company, Kalamazoo, Michigan.

† Obtained from the Drew Company, Boonton, New Jersey.

‡ Mazola Corn Products Refining Company, Argo, Illinois.

§ Lipomul-I.V., Upjohn Company, Kalamazoo, Michigan. This emulsion contains 15% purified cottonseed oil, 1% purified soybean phosphatides, 0.3% Pluronic-F68, and 5% dextrose to volume.

¶ Furnished by the Upjohn Company.

|| Hormel Institute, Austin, Minnesota.

° Prepared by Dr. Walter Gensler from oleic acid-1-C^{14} previously described.[11]

TABLE II

Human Conjunctival Cell Multiplication in the Presence of Various Fat Emulsions

Expt. No.*	Days with substrate	Preparation		Cell population (cells/ml × 10^3)
		No.	Conc. (ml/100 ml medium)	
1	7	3	0.0	545
			0.1	486
			0.5	425
2	5	3	0.0	68
			0.1	65
			0.5	70
		10	0.3	74
		8	0.3	64
3	4	9	0.0	88
			0.3	42
			0.5	37
			1.0	40
		5	0.1	18
			0.3	10
			0.5	10
		6	0.1	30
			0.3	24.5
			0.5	30.5
			1.0	35.5
		5 + 8 (1:1)	0.1	30
			0.3	24.5
			0.5	30.5
			1.0	35.5
		4	1.0	67

* Comparisons of actual cell counts should be made only within experiments, not between experiments, since different absolute cell populations were purposely chosen in the various experiments.

TABLE III

Human HeLa Cell Multiplication in the Presence of Various Fat Emulsions

Expt. No.*	Days with substrate	Preparation		Cell population (cells/ml × 10^3)
		No.	Conc. (ml/100 ml medium)	
4	7	1	0.0	199
			1.0	270
			5.0	231
			10.0	206
	7	2	1.0	285
			5.0	231
			10.0	246
5	6	—	—	177
		10	0.3	169
		8	0.3	200
		3	0.1	186
		3	0.5	176
6	5	3	0.0	21.5
			0.1	24.5
			0.5	17.5
			1.0	14.5
			5.0	19.5
7	5	3	0.0	527.5
			0.1	627.5
			0.2	628.5
8	7	3	0.0	160.5
			0.1	125
			0.3	147
			0.5	109
9	6	—	—	101
		4	0.3	99
		5	0.3	50
		5 + 8 (1:1)	0.3	80
		9	0.3	95
		6	0.3	65
		7	0.3	78.5

* Comparisons of actual cell counts should be made only within experiments, not between experiments, since different absolute cell populations were purposely chosen in the various experiments.

was used without carrier and radiopalmitin[11] was dissolved in nonradiotriolein. The experiments with cells were conducted by incubating the radioactive materials with roller tube cultures containing approximately 2 × 10^5 cells and 1 ml of Eagle's medium containing 10 per cent horse serum. The tubes were equipped with glass ampules containing filter paper,[6] and after 18 to 23 hours of incubation, 0.2 ml of 10 per cent KOH was introduced into the filter paper by means of a syringe and the tubes were incubated for an additional 45 minutes. A 0.1 ml of 10 per cent H_3PO_4 was added to the medium by means of a syringe. After 15 minutes, the KOH ampule was removed and assayed for C^{14} by previously described procedures.[12]

[11] New England Nuclear Company, Cambridge, Massachusetts.

RESULTS AND DISCUSSION

Within the limits of these short-term experiments, the presence of fat emulsion did not greatly alter the net multiplication of either human HeLa or conjunctival cells. The emulsions and related preparations tested,

TABLE IV

Conversion of Glyceryl Triolein-1-C^{14} and Glyceryl Tripalmitin-1-C^{14} to $C^{14}O_2$ by Human Cells of the HeLa and Conjunctiva (Chang) Strains

Expt. No.	Radiosubstrate	Cell strain	Total radiosubstrate (mg)	Total no. cells (× 10^3)	Radioactivity recovered as $C^{14}O_2$	
					Total	Per 10^3 cells
1*	Glyceryl triolein-1-C^{14}	Conj.	1.25	4,670	10,365	22.0
		HeLa	1.25	2,500	10,490	42.0
2†	Glyceryl triolein-1-C^{14}	Conj.	0.25	125	511	4.1
			0.25	125	743	5.9
			0.5	125	1,880	15.0
			0.5	125	1,084	8.7
			0.5	125	400	3.2
		HeLa	0.25	300	2,555	8.5
			0.25	300	1,580	5.3
			0.25	300	890	3.0
			0.5	300	1,540	5.1
			0.5	300	1,300	4.3
			0.5	300	1,092	3.6
3‡	Glyceryl tripalmitin-1-C^{14}	Conj.	0.16	16	231	14.0
			0.16	18	250	14.0
			0.31	26	379	15.0
			0.31	30	190	6.3
			0.31	24	289	12.0
		HeLa	0.31	15	173	16.0
			0.31	16	509	32.0
			0.31	13	287	22.0

* Based on results with 1 stock bottle of each cell strain. Total fluid volume per bottle was 10 ml and total activity was 3.18×10^5 counts per minute (cpm).

† 1.0 mg of triolein furnished 2.54×10^5 cpm. Cell counts given are the mean of four comparable tubes on which no radioassays were performed.

‡ 0.3 mg of tripalmitin furnished 1.73×10^5 cpm. The radioglyceride was dissolved in 0.7 mg of triolein.

given in Table I, represent a rather diverse spectrum of lipids. These were chosen primarily to ascertain whether or not major changes in unsaturation and chain length (Prep. No. 1 vs. Nos. 2, 3, and 4), type of emulsifying agent (Prep. Nos. 2, 3, and 4 vs. No. 5), and so forth, would result in arrested multiplication and cell destruction. In no instance did the emulsions stabilized with Pluronic-F68 cause any adverse effects (Tables II and III), and the fat-free Pluronic preparation (Prep. No. 8, Table I) was also without effect. In connection with the lack of toxicity of this stabilizer may be mentioned the fact that even a final concentration of 1 per cent of Pluronic-F68 in Eagle's medium did not affect the appearance or multiplication of either human HeLa or conjunctival cells.

Emulsion No. 5 and No. 6, containing 2 per cent phosphatide, appeared to depress net multiplication to some extent (Expt. 4, Table II; Expt. 9, Table III), even at the lowest concentration of emulsion tested. Surface and interfacial tension measurements were not done, so the possibility of effects mediated through a reduction in such forces remains. A difference between the two stabilizers employed which is pertinent to the present discussion is that of chemical and enzymatic stability. Phosphatides have various kinds of ester bonds and are therefore labile to hydrolysis, yielding such diverse products as lysolecithins and fatty acids. Such materials could well give toxic manifestations. Pluronic-F68 on the other hand, being a polyether, is fairly inert to cleavage agents present in the systems studied. If any peroxides are formed, they are evidently not injurious. In related studies

use had been made of Pluronic-F68 labeled in the ethylene moiety with C^{14}. The incubation of this material with either HeLa or conjunctival cells has yielded no $C^{14}O_2$, indicating lack of metabolism of the Pluronic-F68. Current in vivo experiments with dogs have shown no alteration in the molecular weight or structure of the Pluronic-F68. Thus, it can be concluded that this material is metabolically inert. No such studies have been done with labeled phosphatides. It should be pointed out that in several experiments the cells grew in a lacy pattern, but no consistent correlation with type of stabilizer or oil could be drawn.

In order to determine whether or not emulsified triglyceride could be used by these human cells, initial studies were done in which the turbidity of the medium was used as the criterion of utilization. No consistent decrease in turbidity upon incubation with the cells could be obtained. Therefore, studies were done with C^{14}-tagged triglycerides. As shown in Table IV, both radiotriolein and tripalmitin were converted to $C^{14}O_2$. In a 24-hour period, approximately 20 μg of triolein, or 28 μg of tripalmitin, was oxidized per 1×10^6 cells. It must be remembered that nonradioactive triolein was also present in the tripalmitin emulsion. Since the caloric requirement of these cells is still unknown, it is not possible to calculate the percentage of the caloric requirement furnished by these triglycerides. The extent to which lipids normally present even in dialyzed serum are utilized by such cells is not known, but it would appear that the capacity to utilize triglyceride and fatty acid is available. It is, of course, possible that enzymatic action of the serum, such as lipolysis, may convert some of the triglyceride to fatty acid and mono- and diglycerides and that these products are substrates for the cells. Further experimentation is necessary to resolve this point. In any event, these human cells can oxidize oleic and palmitic acids to carbon dioxide.

SUMMARY

Two types of fat emulsions have been studied in tissue culture of human HeLa and conjunctival cells. Soybean phosphatides or a synthetic material (Pluronic-F68) was used as the emulsifying agent. Cell multiplication was unaffected by the presence of Pluronic-stabilized emulsions and appeared to be somewhat decreased with a 2 per cent phosphatide-stabilized emulsion. Emulsions of glyceryl triolein-1-C^{14} and glyceryl tripalmitin-1-C^{14} were converted to $C^{14}O_2$ by both kinds of cells.

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The American Diet—Past and Present

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DURING the past decade there has been increasing interest in the possible relationship of food to the incidence of certain diseases. Observations during both world wars stimulated epidemiologic studies in this field. Changes in disease rates during World War II have been associated with alterations in the food available during that period. A decrease in the incidence of multiple sclerosis and atherosclerosis was reported in Norway, Sweden, and Finland.[1] In Denmark, where total fat intake may have decreased but butter fat consumption has increased, there was a decrease in tuberculosis, but deaths from arteriosclerosis remained about the same.[2] Admissions of hypertensive patients to hospitals decreased during the siege of Leningrad when total food supply decreased.[3] While these observations are of interest and have led to a great deal of speculation, care must be exerted in assessing any one factor as the principal or sole agent responsible for changes in mortality and morbidity rates during periods of national emergency.

Since coronary artery disease is a leading cause of death in the United States and in many other countries with similar standards of living, the role of nutrition in its etiology is being studied intensively. A great number of specific nutrients and foods have been implicated as possible contributing agents. Epidemiologic studies have shown an association between the availability of animal fat and coronary artery diseases.[4] Others have debated the importance of this relationship on the grounds that there has been a selection of countries and not all countries with available information were included.[5] Whether coronary artery disease had increased in any degree of magnitude over the last thirty years or so has also been questioned.[6] Nevertheless, whether or not coronary artery disease has increased markedly, it is a most important cause of death, and all facets of its etiology and control should be studied. This has led nutritionists to seek information concerning eating practices in countries with contrasting death rates of coronary artery disease, to investigate regional differences in dietary intake, and to observe changes that have occurred in the American diet. The purpose of this paper is to scrutinize the available literature for information on past and present food practices.

PAST PRACTICES

Food patterns of a country are dependent upon agricultural resources, purchasing ability, technical advances, and cultural patterns. In the United States there has always been an abundance of available food. Calla Van Slyke presented a review of food consumption in this country from 1630 to 1940.[7] Information was gleaned from diaries, ledgers, old account books, and letters. She reported that it was notable throughout the history that reports of food abundance are more common than reports of scarcity. The authors quote a diet recommended in 1872 for a laborer's family of four: On a per capita basis the food allowance included a yearly quota of 38 pounds of butter, 25 pounds of lard, 67 quarts of milk, 250 pounds of meat, 50 pounds of sugar, and 12.5 pounds of coffee. Compare this to the amounts of food available in 1955 (Table II) and it would seem that the Massachusetts Bureau of Labor, which made the

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recommendations, was indeed most generous in some of its items.

In 1894, W. O. Atwater wrote of the errors in our food economy in the Farmer's Bulletin No. 23, published by the United States Department of Agriculture:[8]

> The most remarkable thing about our food consumption is the quantity; for comparable occupations the Americans ate more than the Europeans. German professional men in their quiet, but active and successful intellectual work at home are amply nourished on 2700 calories or less. How happens it that men of mental rather than muscular occupation here consume food with 4000 calories or more?
>
> I think the answer to this question is found in the conditions in which we live. Food is plentiful. Holding to a tradition which had its origin where food was less abundant, that the natural instinct is the measure of what we should eat, we follow the dictates of the palate. Living in the midst of abundance, our diet has not been regulated by the restraints which obtain with the great majority of the people of the Old World where food is dear and incomes are small.

PRESENT PRACTICES

During the past fifty years there has been amazing progress in food technology. The improved methods of food preservation through canning, freezing, and now by irradiation plus the expansion of transportation systems have made seasonal variations in food intake less important. These improvements have been responsible for the increased consumption of certain fruits and vegetables. The availability of mixes, prepared dishes, and even complete meals exemplifies the progress made in food processing. The power of persuasion through advertising cannot be underestimated in motivating the housewife to make purchases and changes in the family food pattern.

During World War II a philosophy of the responsibilities of the government for the health of the people through the improvement of staple foods was developed. Hitherto the major responsibility of the government had been the enforcement of the pure food and drug laws as they pertain to cleanliness, labeling, and additives. The iodization of salt was started in some areas in 1924; now four fifths of all household salt is iodized. Staple foods were not enriched or fortified until about 1941. Approximately 60 per cent of all wheat flour is enriched with iron, riboflavin, niacin, and thiamine. Corn meal and rice are enriched. Prior to 1938 no margarine was fortified; now all margarine has 15,000 International Units of vitamin A added per pound. Milk is being fortified with 400 International Units of vitamin D per quart. One leading city dairy reported that 85 per cent of the fluid milk from their plant is irradiated.[9]

There are many products on the market competing with each other for popularity whose claim for priority in the household food supply is that they have been fortified with additional vitamins, minerals, and protein to make them more nutritious. How much of this is needed and what additional and perhaps unnecessary increase this has on food costs is not known. The rapid growth of the vitamin industry and the widespread and often indiscriminate use of these products has also caused some change in the nutritional picture during the past twenty years. Whether the health of the population has improved because of these enrichment and fortification programs is difficult indeed to evaluate.

METHODS OF STUDY

To find what people eat or what they have eaten in the past, three main sources of data are available: (1) the annual compilation of foods available for human consumption prepared by the United States Department of Agriculture, (2) family food consumption studies, and (3) nutritional studies of specific groups or individuals.

The data most widely quoted when one hears of changes in dietary intake are the tables of the Department of Agriculture entitled "Food Available for Human Consumption."[10] These data are described as residuals from data on production and utilization. From the annual supply of each food are deducted animal feed and seed use, industrial use, other nonfood use, exports, and shipments. The consumption data go back to 1908. The term "consumption" does not mean "food eaten," but "food available at retail level." The data are not adjusted for cooking waste, kitchen losses, or food used in feeding domestic animals. The data are reported in national averages and do

TABLE I

Nutritive Value of Diets Per Person in the United States*

Year	Calories	Protein (g)	Fat (g)	Carbohydrate (g)	Calcium (g)	Iron (mg)	Vit. A (I.U.)	Thiamine (mg)	Riboflavin (mg)	Niacin (mg)	Ascorbic acid (mg)	Calories from fat (%)	Calories from carbohydrate (%)	Calories from protein‡ (%)
1910	3,500	101	123	498	.84	15.3	7,000	1.63	1.86	17.8	107	32	57	11.5
1920	3,280	93	122	460	.88	14.9	7,400	1.53	1.86	16.2	107	33	56	11.3
1930	3,450	92	134	477	.90	14.3	7,800	1.55	1.89	15.9	108	35	55	10.6
1940	3,340	92	142	432	.96	14.8	8,200	1.55	1.95	16.5	122	38	52	11.0
1950	3,250	95	144	401	1.03	17.1	8,200	1.90	2.31	19.4	112	40	49	11.7
1955	3,220	96	148	386	1.04	16.4	7,400	1.85	2.34	19.7	108	43	48	11.9
1955†	3,200	103	155	350	1.15	17.6	8,540	1.56	2.70	18.7	106	44	44	12.8

* Compiled from "Supplement for 1956, Consumption of Food in the United States, 1904–1952," Agricultural Handbook No. 62.

† Taken from "Dietary Levels of Households in the United States," Agricultural Marketing Service and Agricultural Research Service Report No. 6.

‡ The factors 4, 9, 4 were used to figure calories from fat, protein, and carbohydrate for the calculation of the percentages.

not show regional, racial, or economic differences.

Information on family food consumption is also available from studies done by the Department of Agriculture. These studies deal with food brought into the household, either purchased or home produced. The largest and most recent of these surveys is the important 1955 series, "Dietary Levels of Households in the United States, and Food Consumption of Households in the United States."[11] This work presents excellent information pertaining to food brought into households. The data are tabulated for regions, urbanization, family size, and income. The data do not take into account waste, cooking losses, or food practices of individuals.

Dietary data of individuals are usually restricted to small groups of particular populations, such as pregnant women, school children, or factory workers. The surveys may be done for a variety of purposes, such as to study diet in relation to physical condition or to establish base lines for educational programs. The information may be collected by weighing all food and drink, by keeping food lists, and by interviewing the subject.

FINDINGS

Using the annual compilation of foods available, Table I has been prepared.[10] Approximately 25 more grams of fat are available (225 calories) in 1955 than in 1910. Carbohydrate has decreased by 100 g (400 calories). If 1940 and 1955 figures are compared, fat has increased by 6 g and carbohydrate has decreased by 46 g. Actual grams of protein have remained essentially the same. However, the percentage of protein from animal sources has increased. Table II, also prepared from food consumption data,[10] gives some specific food available at the retail level per capita on a yearly basis.

The difference in food availability from 1910 to 1955 can be expressed as pounds change, percentage, or as change in daily amounts on a gram basis. Actual intake of butter has decreased 50 per cent; however, total milk fat has decreased only 8 per cent, or about 3 g per day. Lard has decreased $2^1/_2$ pounds per person per

year, or 3 g per day. Margarine increased by 406 per cent, which means an increase of about two teaspoons daily, and oil consumption increased in approximately the same magnitude. White flour decreased 113 g per person per day, or, placed on a bread basis, approximately five slices less per day. Sugar increased approximately 1 ounce per day. We are eating one potato less daily and one more serving of citrus fruit. When one considers waste, distribution among the individuals of the family, day-to-day variations in the diet, and losses in cooking, one realizes the difficulties in relating this type of information to the increase or decrease of any disease.

It is also seen in Table I that the 1955 food consumption figures as determined by the household survey agree in the main with the type of information in the first part of Table I.[11]

Studies on food waste and losses in food preparation have been limited and have been undertaken primarily in large institutions. In a study of food purchased and served to army recruits stationed at the University of Missouri, it was reported that 18 per cent of the calories were lost, and most of this was fat.[22] Fat loss was 50 per cent from food purchased to food served (this did not take into consideration plate waste). The food as purchased provided 216 g of fat per day, and as served provided 108 g of fat. This changed the percentage of calories available from fat from 43 per cent to 34 per cent. While losses in home preparation would not necessarily be similar to those in institutions, investigations of these losses should be made when accurate data on food consumption are needed. Knowledge of cooking practices may be important; broiling of meats has become a common method of cooking, and the use of the fat in the broiler will vary within households.

Food tables are prepared by averaging samples. The tables may show considerable variation. The differences in these tables may cause overestimation or underestimation of the fat eaten; for example, 100 g cooked porterhouse steak contains 23 g protein and 27 g fat (according to Agriculture Handbook number 8).[13] These figures were based on meat of medium fatness, and adjusted for moderate amounts of trimming. In the publication "The Nutritive Value of Cooked Meat," the nutrients in porterhouse steak have been calculated for lean, marble, and fat portions.[14] The lean portion contains 27 g protein and 8.1 g fat; the marble portion contains 23.6 g protein and 22.7 g fat, and the fat portion contains 4.8 g protein and 83 per cent fat. From this it is obvious that if the meats used in an area are carefully trimmed retail cuts, while the figures used in estimating nutritive value are from wholesale cuts less carefully trimmed, then the estimation of the diet will tend to overestimate fat and underestimate protein.

The consumption of alcohol has been omitted in many dietary surveys. Because of attitudes, it is often difficult to obtain reliable information. Jellinek has published information on the per capita consumption of alcohol from 1850 to 1945.[15] The consumption as expressed in wine gallons was 2.05 in 1850 and 2.09 in 1945. During this period, the amount of beer consumed increased markedly, and the amount of spirits per capita decreased by 50 per cent. This could be interpreted to mean that more people are drinking some type of alcoholic beverage, but fewer people are consuming large amounts of spirits.

The question of alterations in the fatty acid content of the diet is pertinent because of reports of the effectiveness of lowering serum lipids with formula feedings of diets high in fat (40 per cent of calories as fat) but high in polyunsaturated fatty acids. From the food consumption data which revealed 25 g more fat available in 1955 than in 1910, McCann estimated that this could approximate an increase of 5 g of saturated fatty acids, 17 g of monounsaturated (oleic) fatty acids, and 1 g of polyunsaturated fatty acids (mostly linoleic).[16] The estimation of fatty acids in the American diet has been published in the Household Food Consumption Survey of 1955.[17] Approximately 18 per cent of the total calories were from saturated fatty acids, 18.5 per cent from oleic acid, and 4.5 per cent from linoleic acid. Regional differences were slight.

A compilation of a few dietary studies of groups of males during a period of approximately half a century is given in Table III. The basis for the selection of the studies was

TABLE II
Average Amounts of Specific Foods, or Food Groups Available for Human Consumption

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Year	Butter (lb)	Total milk fat (lb)	Lard (lb)	Margarine (lb)	Salt pork, bacon (lb)	Shortening (lb)	Eggs (no.)	White flour (lb)	Cane and beet sugar (lb)	White potato (lb)	Total carcass meat (lb)	Total citrus fruit (lb)	Coffee (lb)
1910	18.3	29.7	12.5	1.6	17.4	8.0	306	214	75.4	198	146.4	17.8	9.0
1920	14.9	28.9	12.0	3.4	17.8	7.6	299	179	85.5	140	136.0	26.0	11.5
1930	17.6	32.1	12.7	2.6	18.8	9.8	331	171	109.6	132	129.0	32.2	12.3
1940	17.0	32.5	14.4	2.4	20.6	9.0	313	155	95.7	123	142.4	67.1	15.3
1950	10.7	29.4	12.6	6.1	19.4	11.0	331	135	100.8	106	144.6	73.1	16.0
1955	9.0	27.3	10.1	8.1	18.7	11.5	371	123	97.5	106	162.8	91.0	16.5
1910–1955 CHANGES	− 9.3	− 2.4	− 2.4	+ 6.5	+ 1.3	+ 3.5	+ 65	− 91	+ 22.1	− 92	+ 16.4	73.2	+ 7.5
% CHANGE	−50	− 8	−19	+406	+ 7	+43.7	+ 21	− 42	+ 29	− 46	+ 11	+411	+83
CHANGE DAILY IN GRAMS	−11	− 3	− 3	+ 8	+ 2	+ 4	+ 10	−113	+ 27	−114	+ 20	+ 20	+ 9.3

Compiled from "Supplement for 1956," from "Consumption of Food in the United States, 1909–1952," Agricultural Handbook No. 62.

TABLE III

Dietary Studies of Males, 1896–1958

Year of study	Type of subject	No. subjects	Method	Calories	Protein (g)	Fat (g)	Carbohy. (g)	Calories fr. fat %	Calories fr. carbohy. %	Calories fr. protein %	Ref. No.
1896–1904	Crew	50	Weighed, waste deducted	4,085	155	177	440	39	43	15	18
1896–1904	Football team	23	Weighed, waste deducted	7,885	270	416	710	47	36	14	18
1896–1904	College club	Total of 15 clubs	Weighed, waste deducted	3,695	107	148	459	36	50	12	19
1896–1904	Mechanics' club	13	Weighed, waste deducted	3,382	118	145	374	39	44	14	19
1896–1904	Professional men	9	Weighed, waste deducted	3,420	107	129	437	34	51	13	14
1896–1904	Laborers (low income)	19	Weighed, waste deducted	3,030	106	117	367	35	49	14	19
1896–1904	Maine lumberjacks	50	Weighed, waste deducted	8,083	164	387	982	43	49	8	20
1943	White, 21 years†	228	Record	2,581	82	103	336	36	52	13	21
1943	White, 16–20 years	40	Record	2,723	87	108	334	36	49	13	21
1943	Colored, 21 years†	98	Record	2,005	65	71	291	32	58	13	21
1943	Colored, 16–20 years	22	Record	2,065	64	71	285	31	55	12	21
1945	Navy mess	Average servings	Weighed, waste deducted*	3,379	120	160	362	43	43	14	12
1946	Army students	Average of 21 meals	Weighed meals	3,692	155	109†	516	27	56	17	22
1955	Age 65†	31	Interview	2,458	87	106	279	39	45	14	23
1956	Factory workers	189	Interview	3,502	120	159	397	41	45	14	24
1956	College professors	20	Interview	2,460	92	123	246	45	40	15	24
1957	College students	156	Record	2,960	114	128	335	39	45	15	26
1957	Soldiers, age 18–25	100	Weighed	2,669	150	162	396	40	43	16	25
1958	Age 14–16	107	Record	2,199	72	80	291	33	53	13	27
1958	Age 14–16	63	Record	2,516	85	104	306	37	47	13	27

* Snacks added. † Ether extract

that the subjects were grouped for age and occupation, and the data were reported in calories (protein, fat, and carbohydrate). Many surveys could not be reported in the table as studies made after 1920 often reported calories, protein, and various minerals and vitamins with no estimation of fat and carbohydrate. While the studies in Table III were conducted by several methods and thus may not be entirely comparable, they can be used for the purpose of this paper, which is to explore trends of eating practices. It is apparent from the data of each survey reported that at any period of time covered by Table III groups of Americans have been eating diets considered high in fat, 40 per cent or more calories coming from fat. And at any time, groups will be eating diets of more moderate fat intake—30 to 40 per cent. It seems improbable that with the vast agricultural resources of this country groups of people would be found who would be eating diets as low in fat as are reported from Japan, Spain, and parts of Italy.[4]

The kinds of fats used in the early studies were primarily butter fat, lard, and salt pork. The only shortening mentioned was "cottolene." There was little or no oil reported. In contrast, the men in the Italian-American and college professor groups studied in 1955–1956[16] used a large variety of fats—butter, margarine, various oils, and many varieties of shortenings. As would be expected, the fruits and raw vegetables were limited by seasonal factors in those early days. Animal protein was generous during the entire period.

SUMMARY

A characteristic of the American dietary that has persisted throughout years has been its abundance. Animal protein, animal fat, and total calories have been high on a per capita basis when compared to many other countries of the world. At any period of time, high fat and moderate fat intakes can be found in groups of the population. It is doubtful that segments of this population would be or have been eating diets low in fat for any period of time.

The change that has occurred in food consumption as judged by the food availability tables has been a marked reduction in the use of cereals and potatoes. Sugar consumption has increased since 1910, but has remained fairly stable since 1933. There has been some change in the amount of fat available and in the variety of fats. The consumption of margarine, oils, and hydrogenated shortenings has increased. Butter consumption has decreased, but that of butter fat has remained about the same.

Studies on waste have been inadequate. There has been much written about the waste of food in America; however, few careful studies of waste in the American home have been made. Whether the increased availability of 25 g of fat since 1910 means an appreciable increase in fat consumed has not been determined. The estimation of fat in meats from food tables will vary according to the table used.

Enrichment and fortification of staple products have been a development primarily of the past twenty years.

Perhaps the most noticeable change in the diet has been the increase in variety of foods and the lessening of seasonal differences in food availability. This has been due to improved transportation, advances in the technology of food preservation, and the influence of very effective advertising methods which persuade the housewife to try new products.

The interpretation of the food availability data and family consumption data as food eaten cannot be entirely justified, as both methods are reported in averages and conceal the variations of individuals. Care must be taken to avoid considering diet, or particular changes in diet, as the sole cause of change in disease rate. Diet should be considered in conjunction with other factors, which may be activity, stress, and genetic or other variables. More information is needed on food intake of specific groups in conjunction with these factors; in addition, more reliable methods should be devised for studying food intake and losses in food preparation and in cooking.

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Diet Therapy

Bookshelf on Nutrition and Diet Therapy

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NUMEROUS publications relating to nutrition and diet therapy appear monthly, and the busy practitioner finds it difficult to keep up with them. When materials ranging from reports on vitamin research to reviews of special diet cookbooks are presented, maintaining an up-to-date bookshelf on nutrition and diet therapy becomes a problem. An attempt has been made to evaluate some of the current literature that might be of interest to the physician and layman.

Since advances in these fields are many and rapid, only recent publications are included, although many earlier ones do have merit. Those listed are but a few out of the wealth of materials available today. An attempt was made to choose those that are most representative of the available supply.

NUTRITION PUBLICATIONS FOR THE PHYSICIAN

Information about nutrition ranging from simple presentations through concise compilations on any one topic as well as detailed research reports is available. Books, booklets and periodicals may be obtained from numerous sources to fill many varying needs.

NUTRITION BOOKS AND TEXTS

If "getting up to date in a hurry" is what is desired, *Present Knowledge in Nutrition*,[1] a brief (128-page) but excellent publication of the Nutrition Foundation is a good choice. A more detailed discussion of each nutrient together with principles of feeding for all age groups can be found in the *Handbook of Nutrition*[2] published by the American Medical Association.

Four widely used college textbooks may be recommended for their careful treatment of the various facets of the nutrition picture. All contain food tables and discussions of the nutritive needs of the many population groups. They are easy to read, lucid, and relatively short.

The newest of these, *Basic Nutrition*[3] by McHenry, a Canadian, is oriented to both Canada and the United States and deals in some detail with world health problems of nutrition. Chaney's *Nutrition*,[4] a standard college text, is very clear in its presentation. *Chemistry of Food and Nutrition*[5] written by Sherman approaches nutrition through chemistry, and *Foundations of Nutrition*[6] by Taylor, McLeod, and Rose gives good coverage to historical developments in the field.

For those interested in books which strongly stress detail, the *Annual Review of Biochemistry*[7] and the *Annual Review of Physiology*[8] are excellent selections. Both report very completely on the latest advances in their respective fields and have an advantage of up-to-dateness because of the annual editions. Another recommendation for extensive recountings on nutrition research is *Biochemistry and Physiology of Nutrition*,[9] a two-volume work by Bourne and Kidder.

Information both wide in scope and minute in detail will be found in *The Vitamins: Chemistry, Physiology, Pathology*[10] by Sebrell and Harris, the first of a proposed multiple volume

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work. Dr. Leslie Harris has written a book presenting the newer knowledge of vitamins, *Vitamins: A Digest of Current Knowledge*[11] which along with excellent presentation has an advantage of brevity.

The ever-present problem of weight control is discussed in a thought-provoking manner in *Weight Control: A Collection of Papers Presented at the Weight Control Colloquium.*[12] Through articles written by investigators well known in the field, the niches of various disciplines in the picture of weight control are brought into focus. No cure-all answers here; but many provocative questions are advanced and discussed.

In a collection of abstracts entitled *Nutrition Sourcebook,*[13] Byrd attempts to give a representative sample of current professional journal literature in nutrition in the past ten years. It is a very interesting book to read, although a great deal of interpretation on the part of the reader is required.

PERIODICALS

Really current information on nutrition is best realized by reading of the various journals. Several journals are devoted entirely to nutrition reporting, including THE AMERICAN JOURNAL OF CLINICAL NUTRITION[14] from which much help of a very practical nature in diet planning may be obtained. The *Journal of Nutrition*[15] contains original reports of investigations in animal and human nutrition. *Nutrition Reviews*[16] brings together in a concise manner the results of the various studies in review-type articles.

The Council on Foods and Nutrition of the American Medical Association from time to time publishes comprehensive review articles on diet in normal and therapeutic nutrition in the *Journal of the American Medical Association.*[17] The *Journal of the American Dietetic Association*[18] contains many articles which give practicable plans and regimens and useful information on nutrition and diet therapy. The *American Journal of Public Health*[19] is another periodical that frequently contains articles dealing with nutrition.

COMMERCIAL HELPS

Many private companies and educational units of some industries publish free materials which are both objective and informative. Well-known authorities contribute materials to many of these publications.

The Borden Review,[20] published monthly, contains comprehensive review articles of timely topics concerning nutrition. *Food and Nutrition News,*[21] a National Livestock and Meat Board organ, *Nutrition News*[22] and *Dairy Council Digests,*[23] both sponsored by the National Dairy Council are monthly newsletters containing current information about nutrition.

Nutritional Data,[24] an H. J. Heinz Company publication, is a small book containing brief digests of knowledge of various nutrients plus food tables. *Vitamin Manual,*[25] published by the Upjohn Company, gives well-illustrated coverage of the present knowledge of the various vitamins.

GOVERNMENT PUBLICATIONS

The *Recommended Dietary Allowances*[26] prepared by the Food and Nutrition Board of the National Research Council and currently under revision is a widely used source of nutritional requirements for all age groups. Brief discussions of the different nutrients and how the allowances were determined are included.

DIET THERAPY PUBLICATIONS FOR THE PHYSICIAN

It is usually difficult, if not impossible, to divorce diet therapy from normal nutrition. Consequently, many books and other publications already mentioned, while concentrating on nutrition, do also treat of diet therapy. Conversely, many of those covered in this section also touch on normal nutrition.

DIET MANUALS

Most physicians find that quick specific helps in meal planning for various therapeutic patterns are invaluable. Diet manuals are to be recommended highly for this purpose. While they usually include little or nothing in the way of principles behind the use of a specific diet, they give useful outlines and meal patterns. These manuals are intended to serve as guides in meal planning, not as lists for diet teaching. Patient instruction should be individualized and flexible.

Three especially good manuals are those of Grace-New Haven Community Hospital,[27]* Columbia Presbyterian Hospital[28]† and the Mayo Clinic.[29] Single copies of the first two are available from the addresses listed below.

HANDBOOKS

If a concise presentation of the principles and calculation of a therapeutic diet is desired, *The Handbook of Diet Therapy*,[30] written by Dorothea Turner for the American Dietetic Association, is very good. It has little in the way of menu plans, however.

Another aid is *Practical Diet Therapy*[31]‡ consisting of reprints from the AMERICAN JOURNAL OF CLINICAL NUTRITION. The composition of numerous therapeutic regimens is covered succinctly, with a practical diet-oriented approach. Sample menus are included.

FOOD COMPOSITION TABLES

Detailed information about food composition must sometimes be obtained, and for this purpose a book of food tables is very valuable. *Composition of Foods—Raw, Processed, Prepared*,[32] a United States Department of Agriculture publication is one of the best known. Two other good choices are *Food Values of Portions Commonly Used*[33] by Bowes and Church and *Bridges' Food and Beverage Analyses*[34] by Mattice. Many of the publications listed in this article also contain food tables.[3–6,24,35–46]

NURSING TEXTS FOR NORMAL AND THERAPEUTIC DIETS

Books that are especially useful in practical menu planning of modified diets are texts intended for use by the student nurse. All of those listed below include simple explanations of normal and therapeutic nutrition in theory and application.

Modern Dietetics[35] by Johnson is the most elementary of the group, but very good. A slightly more detailed presentation is afforded in *Nutrition and Diet Therapy*[36] by Proudfit and Robinson, *Nutrition and Diet in Relation to Nursing*[37] by Krause, *The Art and Science of Nutrition*[38] by Hawley, Carden, and Munves, *Nutrition in Health and Disease*[39] by Cooper, Barber, Mitchell, and Rynbergen, and *Food in Health and Disease*[40] by Mitchell and Bernard.

OTHER DIET THERAPY BOOKS

Several books discussing the detailed principles of dietary treatment in varying degrees of minuteness are available. Although the diet therapy sections include information on practical diet planning, this material is sometimes sketchy and occasionally unrealistic in so far as translating theory into working menus is concerned.

A book that does an excellent piece of work in detailing principles is *Diseases of Metabolism*[41] edited by Duncan. *Clinical Nutrition*[42] edited by Jolliffe, Tisdall, and Cannon is a National Research Council publication covering many facets of nutrition and diet therapy.

Bridges' Dietetics for the Clinician[43] covers completely both therapeutics and normal nutrition. Somewhat less detailed than these is *Therapeutic Nutrition*[44] by Pollack and Halpern, another National Research Council book.

Modern Nutrition in Health and Disease[45] edited by Wohl and Goodhart is also written with a clinical approach. No list of such books would be complete without McLester and Darby's *Diet in Health and Disease*,[46] which is very good on principles, although shorter and containing less detail than some of the others.

MISCELLANEOUS DIET THERAPY PUBLICATIONS

A handy reference for the many aspects of the treatment of diabetes is a paperback publication of the American Diabetes Association, *Diabetes Guide Book for the Physician*.[47] It is well organized and contains a good section on diet including bland diabetic diets and sodium-restricted diabetic diets.

For the theories, indications, contraindica-

* Obtainable from the Dept. of Dietetics, Grace-New Haven Hospital, New Haven 4, Conn. at a price of $1.50.

† Obtainable from Mrs. Nelda Ross, Chief Dietitian, The Presbyterian Hospital, 622 W. 168th St., New York 32, N. Y. at a price of $1.50 (cash). A children's manual is available for $1.25.

‡ Since this paper was submitted for publication, copies of *Practical Diet Therapy* (1955) have been exhausted and will not be available. Announcement will be made in this Journal of publication of another collection of diet-therapy articles.

tions and knowledge surrounding the sodium-restricted diet, *Sodium Restricted Diets—The Rationale, Complications and Practical Aspects of Their Use*,[48] a National Research Council-sponsored publication, is excellent. Food tables and a section on practical diet planning are included.

A book that is difficult to categorize, but which deserves mention is Stern's *Applied Dietetics*[49] by Rosenthal, Baker, and McVey. It is useful in the planning of diets and of special value in its presentation of the techniques of diet teaching.

CHILD FEEDING PUBLICATIONS FOR THE PHYSICIAN

Essentials of Infant Feeding for the Physician[50] by Meyer is a well-written book with a comprehensive bibliography which includes a section on the psychology of child feeding. The current status of nutritive requirements is thoroughly covered in *Modern Trends in Infant Feeding and Nutrition*[51] by Lanman, published by the Sugar Research Foundation. Holt and McIntosh's *Holt Pediatrics*[52] and *The Textbook of Pediatrics*,[53] edited by Nelson, both contain sections on child feeding.

A very comprehensive book on child nutrition is *Roberts' Nutrition Work with Children*[54] by Martin. It covers in detail many aspects of child feeding and contains a wealth of historical and contributory material. *Maternal Nutrition and Child Health, An Interpretative Review*[55] by Toverud, Stearns, and Macy for the National Research Council, reviews material on prenatal and infant nutrition in a thorough and complete manner.

Many of the publications already discussed include information concerning child nutrition. The *Handbook of Nutrition*,[2] any of the college texts,[3–8] some journal articles,[14–19] the *Recommended Dietary Allowances*,[26] *Practical Diet Therapy*,[31] all of the nursing texts[35–40] are to be recommended. Most of the diet therapy texts[41–47] touch upon child feeding in some aspect, and there is a diet manual for children available (see footnote, page 100).

PUBLICATIONS FOR THE PATIENT

Nutrition

Many publications relating to basic nutrition would be of interest to the patient. *Essentials of Nutrition*[56] by Sherman and Lanford and *Nutrition and Physical Fitness*[57] by Bogert are intended for use as textbooks, but could be suggested to the intelligent lay person who desires information about nutrition. Both are interesting, elementary, and easy to read. Any of the nursing texts[35–40] might also be profitably used. These would be doubly useful as all contain material on both nutrition and diet therapy.

Everybody's Book of Modern Diet and Nutrition[58] by Fleck and Munves is a very good paperback book covering basic nutrition and meal planning plus many extras ranging from special diets to food fads. *Food Becomes You*[59] by Leverton is a lively, interesting book written primarily for the homemaker and concerning diets for those with whose feeding she is responsible. Both will be of value to anyone who wants to reduce. A government publication intended for the homemaker is *Nutrition: Up to Date, Up to You*[60] which covers basic nutrition and its translation into meal planning and preparation.

Children who want to learn more about food and nutrition should be introduced to *Food For People*[61] by Riedman. Intended for the elementary school child, it is interestingly written and illustrated. *The Great Nutrition Puzzle*[62] by Callahan and Payne would have great appeal to the teenager who is curious about nutrition, for it delves into the history of this subject as well as covering current knowledge.

Any adolescent girl should like *A Girl and Her Figure*[63] by Leverton, a National Dairy Council publication covering the many aspects of diet and health for the teenager. It is sensible and sound, yet eye-catching, convincing, and well written.

WEIGHT REDUCTION

Reduce and Stay Reduced[64] by Jolliffe would be of interest to the physician as well as the intelligent lay person. The whys of overweight and reducing are discussed in a thought-provoking manner.

Weight control books with less theory and more practical meal planning aids are also available. Dorfman and Johnson's *Overweight Is*

Curable[65] covers the causes, effects, and treatment of overweight in a lucid manner. *Be Slim, Stay Slim*[66] by Winslow features sprightly writing and a well balanced diet pattern.

For the gourmet, Miller's *Reducing Cookbook and Diet Guide*[67] would be helpful. A knowledge of cooking and an interest in working with recipes are prerequisites to using this book.

DIABETES

There are numerous lay publications which would be of assistance to those with diabetes. *Diabetes Control*[68] by Bortz features the exchange system in its diet section and could well be used by the patient and doctor together. Another book with a similar purpose is the *Diabetic Manual*[69] by Joslin. *A Primer for Diabetic Patients*,[70] written by Wilder, could be put to good use by the diabetic patient. All of these books thoroughly cover the treatment of diabetes, dietary and otherwise, and are easy to read.

Meal Planning with Exchange Lists[71] explains the exchange system in graphic fashion and includes some recipes. This booklet, prepared by committees of The American Diabetes Association, The American Dietetic Association, and the Chronic Disease Program of the Public Health Service should be invaluable in helping the patient to translate his prescribed diet pattern into actual foods. Aids in planning a low-sodium diabetic diet[72] and a bland low-fiber diabetic diet[73] are also available. Nine meal plans at various caloric levels have also been devised[74] for use only upon recommendation of the physician. For the convenience of the doctor, these materials[71–74] have been included in *Diabetes Guide Book for the Physician*.[47]

For those desiring a cookbook, *The Diabetic's Cookbook*[75] by Strachan might be recommended. Based on the exchange system, it should serve as a valuable adjunct to other diet instructions.

OTHER THERAPEUTIC DIETS

With the increasing emphasis on the sodium-restricted diet, there has been a demand from the public for more information about and help with this regimen. *The Low Sodium Cookbook*[76] by Payne and Callahan will be helpful to anyone preparing this type of diet. Figures on the sodium and calorie content of each recipe are included. Recently published by the American Heart Association are *Your 500 Milligram Sodium Diet*,[77] *Your 1,000 Milligram Sodium Diet*[78] and *Your Mild Sodium-Restricted Diet*.[79] All include sections on the whys and wherefores of the diet, meal planning, following the diet, and other helpful hints. Diets restricted and unrestricted in calories are discussed. The patient may obtain a copy of the appropriate booklet from the local Heart Association office on the physician's prescription.

Good Food for Bad Stomachs[80] by Jordan and Hibben features recipes for sufferers from ulcers and other digestive disturbances. Since numerous diets fall into these categories, knowledge of what is allowed in the particular regimen of the patient is necessary.

CHILD NUTRITION AND FEEDING

Those with small children and/or an interest in food for children would enjoy and profit from *Feeding Your Baby and Child* by Spock and Lowenberg, a very comprehensive guide with many practical helps and hints for its readers. Virtually all phases of child feeding and nutrition are covered. It is available in both hard-cover[81] and paperback[82] editions.

There are also several very good government publications in this field. *Foods Your Children Need*[83] and *Food for the Family with Young Children*[84] are intended primarily for the homemaker with young children. *Food for Families with School Children*[85] will be helpful to those with older children.

These bookshelf listings could go on until they would more properly be termed bookcase listings and still be far from all-inclusive. Those publications listed are felt to be representative of the sort that would best fill the needs of the physician and his patient.

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Nutrition News

American Board of Nutrition

The American Board of Nutrition will hold the next examinations for certification as a Specialist in Human Nutrition during the week of April 12–18, 1959, in Atlantic City, New Jersey. Candidates who wish to be considered for these examinations should forward applications to the Secretary's office not later than March 1. Application forms may be obtained from the Secretary, Robert E. Shank, M.D., Department of Preventive Medicine, Washington University School of Medicine, Euclid Avenue and Kingshighway, St. Louis 10, Missouri.

Fifth International Congress on Nutrition

The Fifth International Congress on Nutrition, organized by the American Institute of Nutrition and the U.S.A. National Committee for Nutritional Sciences of the National Academy of Sciences, will be held in Washington, D. C., September 1 to 7, 1960, under the auspices of the International Union of Nutritional Sciences.

Dr. C. Glen King, Executive Director of The Nutrition Foundation, New York City, has been named President of the Congress; Dr. Elmer V. McCollum, Professor Emeritus of Biochemistry, Johns Hopkins University, is Honorary President. Dr. Paul György, Professor of Pediatrics, University of Pennsylvania, Philadelphia, is Chairman of the Organizing Committee.

Eminent nutrition scientists from all over the world will participate in the Congress. In addition to papers submitted for the usual scientific sessions, a number of panel discussions and symposia on major problems in the field of nutrition in its broader aspects will be presented.

There will also be scientific and industrial exhibits. Visits to various scientific laboratories and other places of interest will be arranged. A special program for women guests and general entertainment for all registrants are being arranged.

For additional information write to Dr. Milton O. Lee, General Secretary, 9650 Wisconsin Avenue, Washington 14, D. C.

University of Georgia Nutrition Conference

"Better Foods for Better Nutrition," a joint conference sponsored by the Divisions of Foods and Nutrition, and Food Processing of the University of Georgia will be held March 4–6, 1959, at The Center for Continuing Education, University of Georgia, Athens, Georgia.

This Conference is planned for persons engaged in Research, Extension, and Teaching of Foods and Nutrition. Special attention will be given to industrial and institutional problems in processing, handling, and serving foods.

Reviews of Recent Books

Nutrition for You, by Robert S. Goodhart, E. P. Dutton, New York, 1958, pp. 205, $4.50.

The wide general interest in nutrition is reflected in the large number of short books on the subject written for the laity. Often these are of a "sensational" nature; not infrequently promising miracles through dietary manipulations. There has existed, therefore, a great need for an antidote—a book written for the public which presents authoritative information simply, thereby "setting the record straight." Fortunately, such a book is now at hand.

The author, the Scientific Director of The National Vitamin Foundation, writes for the intelligent homemaker in a forthright, factual manner. The style is easy and personal yet does not "talk down" or become annoyingly "chatty."

Outstanding is the emphasis throughout on the point that poor diets exist in this country today chiefly because of ignorance, misinformation, idiosyncrasies, and faddism. In addition, the author's liberal attitude toward nutritional practice is reflected in the absence of rigid standards of nutritional conformity. The book stresses the individuality of dietary habits and the wide range of foods which, if judiciously combined, result in a "proper" diet.

The prevention of nutrient losses in cooking, preserving, and storing food is covered in some detail. Many practical hints are furnished. In addition, a 60-page appendix presents the composition of foods in common household units.

There can be no quarrel with the dietary advice presented in this book. Those who are asked to recommend a "good" book on nutrition would do well to keep this excellent one in mind. S. O. W.

Processed Plant Protein Foodstuffs, ed. by A. M. Altschul. Academic Press, New York, 1958, pp. 955, $26.00.

This book presents a very thorough discussion of the commercial processed plant protein foodstuffs for animals and man. The term "plant protein foodstuff" is interpreted in the usual sense, that is, plant materials unusually high in proteins. The ordinary cereals and vegetables which often supply most of the total dietary protein thus fall outside the scope of the book.

The first thirteen chapters deal with general topics, which include Effects of Heat on Plant Proteins, Use of Processed Plant Proteins as Human Food, Potential Uses of Isolated Oilseed Protein in Foodstuffs, and Supplementation of Plant Proteins with Amino Acids. These selected chapter titles are some which should be of particular interest to those whose primary concern is in human nutrition. Other chapters are devoted specifically to subjects of interest in animal nutrition.

The remaining twenty chapters consider the specific vegetable protein sources—the various oil-seed meals, leaf meals, peas and beans, microbial proteins, algae, etc. The general pattern of each chapter is a discussion of the world production and trade, methods of processing, composition, and uses as food for man and domestic animals. It appears that all of the important or potentially important sources are included. Those who have been wondering where safflower oil comes from will find that the safflower is *Carthamus tinctorius,* is related to the thistle, and grows 18 to 40 inches high. It is one of the oldest oil seeds and has been found in the tombs of the Pharaohs. It was grown at that time as a source of a brilliant scarlet dye for silk. In 1948 over a million acres of safflower were planted in India.

The amount of protein available from these sources is indeed remarkable. For example, the estimated world production of soybeans is estimated as something over 23.8 million tons, with an average protein content of 43 per cent. This, according to a rough calculation, should yield about 10 pounds of first-class protein for each person in the world. Peanuts would yield about half this amount, cottonseed about the same as peanuts, etc. Such figures at least suggest that much of the so-called "protein problem" of man may be due to his stupidity or general cussedness in having likes and dislikes that are not always well designed for efficient use of natural resources.

An evaluation of the "protein problem" per se falls outside the scope of this book. In some chapters there is the familiar inference that a great deal more protein for human nutrition is a serious need. Actually, there is very little evidence of protein malnutrition in adult man anywhere in the world, and the reviewer is willing to hazard the guess that, when all of the evidence is in, the protein deficiency which is evident in malnutrition in children will, like the classic protein deficiency of hunger edema, be shown to be largely caloric undernutrition. Nevertheless, with the ever-increasing world popula-

tion and the inevitable high cost of animal proteins, a greater and more efficient use of foodstuffs of all kinds through direct consumption by man would seem to be inevitable. Thus, the subject matter of this book should become of increasing importance in human nutrition.

The topic of amino acid supplementation is treated in a practical way. Methionine and lysine are the two amino acids which are generally limiting in proteins. The practicality of methionine supplementation in poultry feeding casts a shadow of things to come. A demonstration of need and the relative costs will be the factors which determine how widely this practice becomes in the future. Supplementation with small amounts of synthetic amino acids to improve the biologic value of specific foods or diets is easily possible, but bulk synthesis of amino acids to compete with natural protein sources does not appear to be a likely possibility.

Dean presents, in his discussion, numerous practical examples of the way vegetable protein sources have been prepared in a manner acceptable for human consumption. In other places, examples of "all-vegetable coffee cream, all-vegetable frankfurters," etc., are given. Although there is much that remains to be done, there is no doubt that adequate diets for human beings can be derived from vegetable sources. Perhaps greater utilization will come when less attention is put upon "what people need" and more attention to making foods "that people like." A realization of the extensive data upon protein and amino acid needs of domestic animals makes it clear that our knowledge of the needs of human beings is largely a matter of guesses and it may be argued as to how "educated" these guesses are.

This should be a very useful book for people in many different fields From the standpoint of human nutrition the subject matter is more germane to the problems of the so-called technically underdeveloped areas of the world. It is unfortunate that complete coverage of a topic results in a book of over a thousand pages and a cost that will limit its availability.

D. MARK HEGSTED

Transactions of the 6th Meeting of the International Society of Geographical Pathology. S. Karger, New York, 1958, pp. 522, Sfr. 67.60.

In July, 1957, a conference was held in Paris on geographic pathology, with special emphasis given to peptic ulcers. The transactions of the meeting are presented in this trilingual book. Much of the data is based on vital statistics of countries obtained at varying levels of completeness. In spite of obvious problems in this regard, the amount of data is considerable.

A number of especially interesting papers are included, among them a study on smoking and peptic ulcer, and others which indicate an extremely high morbidity from gastric ulcer in Japan and the prevalence of ulcer among the poor populations of India and the Congo. Several reports on dietary factors in different areas add to the usefulness of these papers.

Those who are interested in the regional differences in disease as clues to etiology, and all interested in the worldwide ulcer problem will find much valuable information in this book. A. E. S.

General Biochemistry, by Joseph S. Fruton and Sofia Simmonds, Wiley, New York, 1958, ed. 2, pp. 1,077, $18.00.

The first edition of this textbook was published in 1953. In the intervening five years so many significant developments in biochemistry have occurred that the publication of a second edition was inevitable.

The book is based on the authors' many years of teaching at Yale University. It is aimed at the serious and rather advanced student and, accordingly, stresses enzyme chemistry and intermediate metabolism. Although not emphasizing medical or clinical aspects it nevertheless presents a comprehensive and thorough review of "pure" biochemistry which, as the authors remind us, the medical student will need to know for an adequate understanding of many problems of human physiology and pathology.

Useful features are the large number of references presented (although without article titles) as footnotes, the many illustrations and a good detailed index.

This may be considered one of the better standard reference works in a highly complex and ever-broadening field. S. O. W.

Ciba Foundation Colloquia on Endocrinology. Volume 12: Hormone Production in Endocrine Tumors, edited by G. E. W. Wolstenholme and M. O'Connor. Little, Brown, Boston, 1958, pp. 351, $9.00.

The induction and function of tumors of the hypophysis, thyroid, adrenals, and sex glands in various species, especially in rodents, are discussed in twelve major and eight short papers. The various facets of histogenesis and function are reviewed by the participants who restate and summarize their views, most of which have been expressed previously. The reader will find adequate and useful information about the progress made in this intriguing area of experimental endocrinology. While all subjects under discussion are of interest to the biologist, pathologist, and endocrinologist, the clinical nutritionist will be primarily interested in the discussion of thyroid and hypophyseal tumors, and find of particular interest those data that refer to dietary factors and to associated obesity. Thyroid tumors, adenomas and carcinomas, may be produced in rats and mice by addition of goitrogens to the diet, or of carcinogens such as acetaminofluorene, or simply by feeding a low-iodine diet without supplements of either goitrogens or carcinogens. Many of these tumors are transplantable. Metabolic studies on animals bearing such tumors are not available. Hypophyseal tumors

may be induced by chemical, radiologic, or surgical thyroidectomy, or by feeding of a low-iodine diet. The neoplasms thus produced are transplantable under certain conditions; they may be nonfunctioning, or they may show thyrotropic, adrenotropic, mammotropic, somatotropic, or gonadotropic activity. Only those produced by ionizing radiation show adrenotropic function of different degree. The changes noted in mice developing the latter tumors or in hosts bearing grafts of such neoplasms are considered to be due to increased activity of gluco- and mineralocorticoids. The most striking effect is obesity. In fasted mice, liver glycogen is markedly increased, lipogenesis is enhanced; increased amounts of fatty acids and of tissue and serum cholesterol are synthesized from labeled acetate; blood sugar values are essentially unaffected; liver glucose-6-phosphatase activity is elevated without changes in live-phosphorylase or hexokinase. Associated with the aforementioned changes is a negative nitrogen balance resulting from replacement of protein by fat. A general index of this and the previous eleven Colloquia on Endocrinology sponsored by the Ciba Foundation added to the volume. This facilitates a quick orientation about the subject matter.

M. SILBERBERG

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable, those of special interest are selected, as space permits, for a more extensive review.

The "Triad Disease" by N. Philip Norman, Lee Foundation for Nutritional Research, Milwaukee, 1958, pp. 216, $4.75.

Etude des Carences Protidiques Observées chez l'Enfant en Pays Tropical: Kwashiorkor by Henri Dupin, Librairie Arnette, Paris, 1958, pp. 169.

Essentials of Therapeutic Nutrition by Solomon Garb, Springer Publishing Co., Inc., New York, 1958, pp. 147, $2.00.

The Birth of Normal Babies by Lyon P. Shean, Twayne Publishers, Inc., New York, 1958, pp. 194, $3.95.

Guide Coprologique pour l'Interprétation Clinique de l'Examen des Selles by J. Tauzin, Masson et Cie, Paris, 1958, pp. 131, 1,250 fr.

Vitamins and Hormones: Advances in Research and Applications, Volume XVI, edited by Robert S. Harris, G. F. Marrian, and Kenneth V. Thimann, Academic Press, Inc, New York, 1958, pp. 437, $11.60.

Nutrition in Health and Disease by Lenna F. Cooper, Edith M. Barber, Helen S. Mitchell, and Henderika J. Rynbergen, J. B. Lippincott Company, Philadelphia, 1958, pp. 734, $6.00.

The Health of a Nation: Harvey W. Wiley and the Fight for Pure Food by Oscar E. Anderson, Jr., University of Chicago Press, Chicago, 1958, pp. 333, $6.00.

Modern Chemotherapy of Tuberculosis by Roger S. Mitchell and J. Carroll Bell, Medical Encyclopedia, Inc., New York, 1958, pp. 109, $4.00.

Streptomycin and Dihydrostreptomycin by Louis Weinstein and N. Joel Ehrenkranz, Medical Encyclopedia, Inc., New York, 1958, pp. 116, $4.00.

Chloromycetin (Chloramphenicol) by Theodore E. Woodward and Charles L. Wisseman, Jr., Medical Encyclopedia, Inc., New York, 1958, pp. 159, $4.00.

Nutrition and Atherosclerosis by Louis N. Katz, Jeremiah Stamler, and Ruth Pick, Lea & Febiger, Philadelphia, 1958, pp. 146, $5.00.

Penicillin by Harold L. Hirsh and Lawrence E. Putnam, Medical Encyclopedia, Inc., New York, 1958, pp. 148, $4.00.

Clinical Endocrinology, ed. 2, by Karl E. Paschkis, Abraham E. Rakoff, and Abraham Cantarow, Paul B. Hoeber, Inc., New York, 1958, pp. 941, $18.00.

Essential Fatty Acids, edited by H. M. Sinclair, Academic Press, Inc., New York, 1958, pp. 268, $9.50.

Food for Better Performance by R. C. Hutchinson, Melbourne University Press (Cambridge University Press, New York), 1958, pp. 102, $2.75.

Recent Progress in Hormone Research: Volume XIV, Proceedings of the Laurentian Hormone Conference 1957, edited by Gregory Pincus, Academic Press, Inc., New York, 1958, pp. 582, $13.50.

Clinical Chemistry in Practical Medicine, ed. 5, by C. P. Stewart and D. M. Dunlop, E. & S. Livingstone, Edinburgh (The Williams & Wilkins Co., Baltimore, exclusive U. S. agents), 1958, pp. 342, $6.75.

Abstracts of Current Literature

LIVER FUNCTION AND NUTRITION

Patients with liver disorders should receive a high level of protein intake with adequate calories provided in a high carbohydrate and moderate fat allowance. Such a diet furnishes the necessary elements for reconstitution of hepatic proteins, enzymes, and glycogen. In advanced states of hepatic dysfunction, utilization of amino acids for protein biosynthesis is impaired so that these factors accumulate in the blood giving rise to the increased production of ammonia and other potentially toxic substances. It may be of interest to measure the ability of the liver to handle ingested protein so that the clinician could determine when the level of dietary protein is becoming hazardous to the patient.

The Plasma Amino Acids in Patients with Liver Failure. F. L. Iber, H. Rosen, S. M. Levenson, and T. C. Chalmers. *J. Lab. & Clin. Med.* 50: 417, 1957.

This paper presents plasma amino acid levels in two normal subjects and in seven patients with liver disease of varying severity. The effect of intravenous sodium glutamate was also noted in two normal subjects and in three of the patients with hepatic coma. The plasma amino acids were usually elevated in severe liver disease but were not always elevated in hepatic coma. Methionine and tyrosine were disproportionately elevated.

Sodium glutamate was metabolized rapidly both in normal subjects and in patients with liver failure. Following the infusion there was a rise in all plasma amino acids, but alanine and glutamic acid showed the greatest increases. A theory is also offered in an attempt to explain the abnormality of elevated amino acid level in patients with liver disease.

K. R. CRISPELL

Effect of Portal Blood Ammonium Concentration of Administering Methionine to Patients with Hepatic Cirrhosis L. T. Webster, Jr. and G. J. Gabuzda. *J. Lab. & Clin. Med.* 50: 426, 1957.

This is a very clear, well planned, and concise study of the effect on portal collateral blood ammonium concentrations of administering methionine to patients with hepatic cirrhosis.

Ten grams of ingested DL-methionine caused increased concentrations of ammonium or a related substance in the portal collateral blood of each of three fasting patients with hepatic cirrhosis. This increase was largely prevented by prior administration of neomycin sulfate. This suggests that "ammonium" is probably formed from methionine and is dependent to some extent upon the integrity of the gastrointestinal flora.

The study was well controlled and would appear to establish the fact that methionine may possibly induce the syndrome of impending hepatic coma in susceptible patients with liver disease. K. R. CRISPELL

Liver disease due to nutritional deficiencies has been accepted as a clinical and experimental fact. The manifestations of hepatic disease on this basis will vary among individuals or in different geographic locations depending upon many conditioning factors including sex, endocrines, physical activity, food consumption, infection, and possible toxic substances encountered in certain diets.

Observations on Hepatic Disease in the Gold Coast: With Special Reference to Cirrhosis. G. M. Edington. *Tr. Roy. Soc. Trop. Med. & Hyg.* 51: 48, 1957.

The author reviews the results of 552 consecutive autopsies in Accra, Gold Coast. The incidence of cir-

rhosis and hepatoma was high, particularly in men. Hepatitis and massive necrosis were more common in women. In one-third of children up to five years old there was a proliferation of fibrous tissue in the portal tracts. The author doubts whether this fibrosis could result from an episode of fatty infiltration, as some cases show fibrosis without fat and others fat without fibrosis.

The reasons are discussed why cirrhosis should be common in the Gold Coast. Among the possible causal factors are malnutrition, malaria, infectious hepatitis, and toxic alkalosis. The author does not think malnutrition as important a cause as it is claimed to be elsewhere in the tropics. Forty per cent of the male cirrhotic livers contained abnormal deposits of iron, but this is not considered an important factor in the production of cirrhosis itself. J. WATERLOW

Bantu Porphyria. A Clinical Study of Sixteen Cases. M. Gelfand and J. D. Mitchell. *Tr. Roy. Soc. Trop. Med. & Hyg.* 51: 62, 1957.

A form of porphyria is found among the Bantu of South Africa and Southern Rhodesia which resembles the syndrome of chronic or delayed cutaneous porphyria in Europeans. Sixteen cases are described in this paper. The chief signs were hyperpigmentation of exposed parts, blistering of the hands, dystrophy of the nails, and excessive amounts of porphyrins and urobilin in the urine. Neurologic disturbances were rare, and so were the attacks of abdominal pain that are typical of acute porphyria. Many of the patients had enlarged livers and abnormalities in their liver function tests. However, in a control series of 56 patients, including cases of hepatic cirrhosis and of pellagra, not one had porphyrinuria. It is therefore concluded that the disorder is not simply a result of liver disease. The suggestion is made that it might be caused by drinking excessive amounts of a kind of native beer called "skokiaan." J. WATERLOW

Increased serum levels of vitamin B_{12} in liver injury induced by carbon tetrachloride represents release of stored vitamin from damaged cells. Similar observations have been made in acute liver injury due to infection or other causes.

Effect of Carbon Tetrachloride Injury on Plasma and Liver Vitamin B_{12} Levels. R. S. Yamamoto, K. Okudo, and B. F. Chow. *Proc. Soc. Exper. Biol. & Med.* 94: 497, 1957.

Administration of carbon tetrachloride increased the serum level of vitamin B_{12} of rats fed with casein in the diet. Within limits of the experiment the level of vitamin B_{12} increased with increasing doses of carbon tetrachloride. No similar increase in serum levels was found in rats previously offered a low vitamin B_{12} diet. Studies with the radioactive vitamin demonstrated higher excretion rates in the urine and feces after the rats were given carbon tetrachloride. These studies suggest that interpretation of vitamin B_{12} levels in serum must take into account the existence of an agent which may temporarily release vitamin B_{12} from storage organs. A low level of vitamin B_{12} may be considered a measure of poor reserve. A high level, particularly in those with abnormally elevated values, need not indicate sufficiency of the vitamin. L. KINSELL

Studies on Copper Metabolism: XXIII. Portal (Laennec's) Cirrhosis of the Liver. C. J. Gubler, H. Brown, H. Markowitz, G. E. Cartwright, and M. M. Wintrobe. *J. Clin. Invest.* 36: 1208, 1957.

Copper metabolism is related to the liver. For example, disorders of copper metabolism have been a constant finding in hepatolenticular degeneration. Therefore, a study of copper metabolism was made in patients with cirrhosis of the liver as well as in normal controls. The results indicate that in some patients with hepatic cirrhosis, significant alterations in copper metabolism occur. The fraction of copper which is "loosely bound" to proteins is increased. There was a significant increase in the "non-ceruloplasmin" fraction of copper in serum, increase in the urine and spinal fluid, and a greater proportion of the brain copper remained in aqueous solution after homogenization.

Following the administration of BAL, the patients with liver disease excreted an average of 87 μg of copper in the urine daily, whereas normal subjects excreted an average of 40 μg. In addition, the mean value for the total amount of copper in the liver of patients with cirrhosis was more than twice as much as that in normal subjects. This elevated total liver copper was found in 12 patients. In 6, however, there was a normal amount of copper. Additional data are presented in this paper.

Alterations in copper metabolism did not seem to be of significance in the pathogenesis of the neuropsychiatric manifestations of hepatic cirrhosis. S. O. W.

The inability of the diseased liver to inactivate estrogens is thought to be responsible for the appearance of spider angiomata, liver palms, gynecomastia, and testicular atrophy. Some of the mechanisms involved in the hepatic metabolism of estrogen are discussed in the following report.

Relationship of Low Protein Diet and Ascorbic Acid in Estrogen Inactivation by Liver. F. D. Vasington, A. Parker, W. Headly, and R. E. Vanderlind. *Endocrinology* 62: 557, 1958.

The influence of protein and ascorbic acid intake on inactivation of estrogens was studied in rats using both in vivo and in vitro technics. In vivo experiments demonstrated that animals on a low-protein diet (8 per cent casein) rapidly lost the ability to inactivate estrone. Restoration of the ability to degrade estrone followed the administration of ascorbic acid. In vitro studies using liver slices from rats fed 8 per cent or 21 per cent casein showed that those animals fed the low-protein diet could not metabolize estrone even in the presence of ascorbic acid or other coenzymes, whereas

livers from animals fed the high-protein diet rapidly inactivated this hormone. It was of interest, however, that liver slices from rats fed the 8 per cent casein diet inactivated estradiol as efficiently as liver slices from rats on the high-protein intake. It is postulated that low-protein diets may adversely affect enzymes responsible for conversion of estrone to estradiol so that this conversion may be the initial step in estrone inactivation. A. B. EISENSTEIN

Apparently lysine is not essential for the regeneration of liver cells, or the availability of this amino acid from other protein sources is adequate for hepatic repair despite its restriction from the diet.

Regeneration of Liver in Lysine-Deficient, Partially Hepatectomized Rats. M. K. Lavers, L. E. Hallanger, and M. O. Schultze. *Proc. Soc. Exper. Biol. & Med.* 97: 621, 1958.

Mature female rats fed lysine-deficient diets are in negative nitrogen balance and show decrease of plasma proteins and abnormal liver composition. This study was undertaken in order to investigate the effect of lysine deficiency on regeneration of the liver. Mature female rats weighing about 20 g were housed individually and fed lysine-deficient diets ad libitum for about two weeks. At that time partial hepatectomy was performed, and the course of liver regeneration was observed. As indicated by dry weight, lipid and nitrogen percentages of dry liver, and percentage of moisture, regeneration in deficient animals was complete and the findings did not differ materially from those obtained in controls fed a complete diet. M. SILBERBERG

Effects of Certain B-Vitamin Deficiencies on Liver Nitrogen in Rats. K. Guggenheim and S. Halevy. *Internat. Ztschr. Vitaminforsch.* 28: 301, 1958.

Rats deficient in either thiamine, pyridoxine, riboflavin, pantothenic or folinic acids have a higher liver nitrogen content per unit body weight than either ad libitum or pair-fed controls. In comparison with animals of equal weight maintained on a full diet offered ad libitum, liver nitrogen was found to be diminished in thiamine-deficient rats, to be increased in riboflavin deficient animals, and not significantly changed in rats deficient in pyridoxine, pantothenic, folic, or folinic acids. AUTHORS

Experimentelle Untersuchungen über Lebernekrose in absolutem Hungerzustand. (Experimental Investigations Regarding Liver Necrosis in Absolute Starvation.) I. Schlicht. *Virchows Arch. path. Anat.* 330: 436, 1957.

Adult male mice weighing 25 to 30 g were kept at 30° C without food. The animals had, however, access to water. Young mice were not used, since they survived only one day or two the state of starvation. Some of the starved animals received 10 to 30 mg methionine or a liver dialysate (Prohepar) intramuscularly. About two thirds of the mice remained alive for more than three days, and about 1 per cent survived about one week. The livers of the animals which had survived three days were congested at necropsy. Slight liver changes consisted of cytoplasmic vacuolation and ballooning of liver cells with deposition of hyaline droplets in the degenerating cells. Moderate damage was indicated by dissociation of liver lobules, pyknosis of nuclei, and small foci of necrosis of central lobules. Severe damage was represented by diffuse massive necrosis. The onset and severity of these changes was not necessarily correlated to the duration of the survival of the animals. Neither methionine nor the liver dialysate had any beneficial effect on the development of the liver lesions in the starved animals. Apparently increased protein metabolism and proteolysis with formation of toxic substances are involved in the evolution of the pathologic liver conditions. M. SILBERBERG

Glucose Penetration into Liver. G. F. Cahill, Jr., J. Ashmore, A. S. Earle, and S. Zottu. *Am. J. Physiol.* 192: 491, 1958.

To further define the action of insulin on liver metabolism, studies were undertaken to determine whether the cell wall could be the site of action, as has been proposed for muscle. Control rats and those injected with varying amounts of glucose were found to have a higher glucose concentration in liver water compared to plasma water with low plasma glucose levels and vice versa with high plasma glucose levels. Radioactive glucose was freely distributed into total liver water in dogs, whether the liver was taking up or producing glucose. Other compounds which entered rat liver cells were fructose, mannose, galactose, sorbitol, mannitol, glycerol, and α-methyl glucopyranoside. Maltose, sucrose, and raffinose were restricted to extracellular fluid. Alloxan-diabetic rats likewise showed free equilibration of radioactive glucose between plasma and liver water. The free permeability of liver to glucose and other small carbohydrates suggests that insulin in liver alters intracellular enzyme functions and not permeability of the cell wall as in muscle. AUTHORS

Liver Pattern in Biliary Hypercholesteremic Xanthomatosis. H. E. MacMahon. *J. Mt. Sinai Hosp. New York* 24: 1024, 1957.

A variety of distinct microscopic changes in the liver is seen in the syndrome characterized clinically by hypercholesteremia, xanthomatosis, prolonged jaundice, and liver enlargement. Details about the gross and microscopic structural changes are presented on the basis of examination of 39 cases. The distribution of regressive changes and of the inflammatory reaction, the relative predominance of degeneration or necrosis of liver cords and the type of cellular response, the distribution of bile pigmentation and bile stasis, the regeneration and pattern of bile duct proliferation and connective tissue growth allow the following grouping of the pathologic changes:

(1) Pericholangiolitic biliary cirrhosis, a group which includes early stages of Hanot's cirrhosis (two cases); (2) congenital acholangic biliary cirrhosis (three cases); (3) acquired absence of interlobular bile ducts; (4) obstructive biliary cirrhosis (four cases); (5) cholangiolitic biliary cirrhosis (five cases); (6) pigment cirrhosis (seven cases); (7) portal-hepatic cirrhosis (eight cases); (8) fibroxanthomatous biliary cirrhosis (nine cases).

In spite of the differences in the structural changes observable under these conditions, a common feature is the occurrence of lipid-laden histiocytes along the sinuses or in the portal connective tissue, indicating a disturbance in lipid metabolism. M. SILBERBERG

HEPATIC COMA

New concepts in nutrition concern the relationship of protein intake and utilization in patients with advanced liver disease. The defect in the disposal of nitrogenous residues of protein degradation results in an elevation of these toxic substances in the blood producing the syndrome known as hepatic encephalopathy. The principal defect causing a rise in blood ammonia content is that of a reduced rate of urea formation through the arginine-citrullene-arginine cycle. Curtailment of protein intake lowers the formation of these nitrogenous residues correcting for a time the symptoms attributed to them.

Cirrhosis of the Liver. Impending Hepatic Coma and Increased Blood Ammonium Concentrations During Protein Hydrolysate Infusion. L. T. Webster, Jr. and C. S. Davidson. *J. Lab. & Clin. Med.* 50: 1, 1957.

This is another in a series of articles on impending hepatic coma and increased blood ammonia concentrations. This study is concerned with the effect of the slow and rapid infusion of protein hydrolysates in patients with cirrhosis of the liver.

The slow infusion of these substances did not appreciably alter either the blood ammonia nitrogen level or the clinical condition of the patient. However, the rapid infusion was associated with a significant rise in blood ammonia nitrogen and the onset of impending hepatic coma (mental confusion and "flapping" tremor). These studies indicate that the ammonium contained both in the hydrolysate and produced by its administration could account for the increased blood ammonium concentration observed.

The authors attempt to explain the difference between slow and rapid infusion rates on the level of blood ammonium. They postulate that the protein hydrolysate contained either an ammonium neutralizing or disposing compound, e.g., arginine. In addition the glutamic and/or aspartic acid might help to prevent the rise in blood ammonia.

This is an excellent study with challenging and interesting theories which the authors hope will be confirmed by other investigations.

K. R. CRISPELL

Glutamic acid and L-arginine have been used as ammonia acceptors to increase the rate of conversion of ammonia to urea through the Krebs urea cycle. While both substances have proved effective in several reported series including those below, an equal number of unfavorable experiences have been reported. It is of interest that a good correlation between blood ammonia levels and clinical findings has not been made.

Effects of L-Arginine on Hepatic Encephalopathy. J. F. Fazekas, H. E. Ticktin, and J. G. Shea. *Am. J. M. Sc.* 234: 462, 1957.

Ten patients with advanced cirrhosis with severe hepatocellular insufficiency and evidences of cerebral dysfunction received 25 to 37 g of L-arginine before and after which cerebral blood flow, oxygen consumption, and blood urea nitrogen were determined. Arterial blood ammonia was measured before and during the three-hour infusion of the amino acids placed in 10 per cent glucose in water. The results of this study disclosed a significant but temporary reduction in blood ammonia after L-arginine. There was no change in cerebral oxygen consumption. Clinical improvement was noted in three subjects during the infusion. In five others, the clinical status improved after ammonia levels had risen to pre-infusion values. Despite the lack of correlation between encephalopathy, oxygen consumption, and ammonia levels in the blood, it would appear advisable to administer those amino acids which reduce blood ammonia to all patients with hepatic coma. C. R. SHUMAN

Glutamic Acid in the Treatment of Hepatic Coma and Its Relationship to Blood Ammonia Levels. N. W. Chaikin and M. S. Konigsberg. *Am. J. Gastroenterol.* 27: 266, 1957.

A total of 22 patients with cirrhosis of the liver and evidence of hepatic coma or pre-coma were treated with glutamic acid. Ten of the patients were in coma and these were given 25 g of sodium glutamate intravenously three times daily until the patient regained consciousness. Twelve patients had neurologic and mental symptoms of pre-coma and these were given 15 g of glutamic acid orally. Patients recovering from coma were given oral glutamic acid in similar doses. The treatment was continued until the mental and neurologic abnormalities disappeared. Of the ten comatose patients, six died in coma, four recovered from the initial episode of coma, but three subsequently died following hemorrhage. Of the 12 patients with pre-coma, 8 of the 12 showed marked improvement with complete disappearance of mental symptoms and neurologic signs. Four patients, despite lowering of the blood ammonia levels, progressed to coma and died.

The authors concluded that glutamic acid and a low-protein diet are useful adjuncts in treating the neurologic complications of hepatic disease in that they delay a fatal termination and may permit the treatment of the underlying liver disease.

In this study there was no attempt to distinguish between patients with relatively more reversible liver coma and those with relatively less reversible coma. Other studies have demonstrated a more favorable effect of glutamic acid in the former group. Although it has been demonstrated that antibiotics will diminish blood ammonium values, the use of such agents in this series is not mentioned. Obviously, such a study will be more valuable when it is possible to include a control series of patients with similar degrees of liver coma for comparison with the treated group.

J. B. HAMMOND

The Serum Neuraminic Acid Distribution. I. Methodology. A. Saifer and S. Gerstenfeld. *J. Lab. & Clin. Med.* 50: 17, 1957.

The authors have made studies on the concentrations of blood ammonia and other nitrogenous substances following the experimental introduction of blood into the stomachs of patients with liver disease and a control group.

Five hundred ml of blood was given by gavage through a Levin tube in a fasting state. The blood ammonia concentration was determined at hourly intervals for three hours and at six and nine hours. The plasma urea nitrogen and non-protein nitrogen were determined also.

It was clearly demonstrated that in patients with liver disease the presence of blood in the gastrointestinal tract was associated with a marked elevation of blood ammonia over that of the control group.

K. R. CRISPELL

METABOLISM IN LIVER DISEASE

Accumulation of fat in liver cell cytoplasm is observed in a wide variety of conditions from obesity to starvation in patients without obvious disease and in anoxic, toxic, infectious, and metabolic disorders. Extensive fatty metamorphosis may be present without any demonstrable functional abnormalities. The origin of hepatic fat in this condition is uncertain; however, it seems probable that it is transported to the liver where utilization is impaired.

Co-enzyme A Content and Fatty Infiltration of the Liver. V. Ferrari. *Acta vitaminol.* 11: 31, 1957.

The problem of the local deficiency of coenzyme A in the pathogenesis of various forms of fatty infiltration of the liver is discussed in the light of the available experimental and clinical data. Fatty liver following partial hepatectomy (as appears from the experimental part of this article) shows a reduced concentration of pantothenic acid, probably due to interference with the synthesis of coenzyme A exerted in the growing tissue by other processes capable of competing for common building materials and energy sources. Decrease of coenzyme A in liver tissue has hitherto been demonstrated in various nutritional and toxic conditions, not all of which are characterized by accumulation of fat: in view of this, it is not specific of the latter alteration as such. However, in the various forms of fatty infiltration it appears to be almost constant (the main exceptions, which may perhaps be explained separately, appear to be the liver of growth hormone treated animals in the experiment, and of "fatty" diabetes in man). This fact, although not decisive in itself, points to a probable pathogenic role of a local coenzyme A deficiency in lipid accumulation in the liver. This conclusion may be useful for purposes of experimental and therapeutic research, particularly when considered along with other factors relevant to the functioning of the fatty acid cycle.

AUTHOR

The use of alcohol by patients with liver disease has been forbidden by most clinicians. The following report requires extensive confirmation over a long period before its conclusion can be accepted.

Response to Alcohol in Chronic Alcoholics with Liver Disease. Clinical, Pathological and Metabolic Changes. W. H. J. Summerskill, S. J. Wolfe, and C. S. Davidson. *Lancet* 1: 335, 1957.

It is generally accepted that there is an association between liver disease, chronic alcoholism, and malnutrition but the relationship has been confused.

The effect of alcohol during treatment of alcoholic liver disease was studied in six men and one woman from 33 to 67 years of age who were heavy drinkers and had generally poor diets. Biopsy of the liver showed portal cirrhosis in six, five of them with fatty infiltration; and one with fatty vacuolization. The patients were given diets of from 2,286 to 2,800 calories and from 25 to 65 g of protein. After a control period, 90 to 120 ml of ethyl alcohol was given during the day in fruit juice to four patients as an addition to the diet and to three after deduction of an equivalent amount of carbohydrate.

Addition of alcohol caused euphoria and an increased appetite. The calories from the alcohol were used and nitrogen balance was not affected. Liver biopsies and function tests revealed no adverse effect. It is suggested that alcohol may have a place in the treatment of alcoholics with liver disease.

F. E. HYTTEN

Vitamin B_{12} has been employed in large doses in the treatment of liver diseases. However, high serum levels of the vitamin have been repeatedly demonstrated in these conditions suggesting that there is probably no basis for this type of therapy.

The Effect of Liver Disease on Serum Vitamin B_{12} Concentrations. P. N. Jones, E. H. Mills, and R. B. Capps. *J. Lab. & Clin. Med.* 49: 910, 1957.

The authors present studies on the serum vitamin B_{12} levels in 36 patients with liver disease. Serial determinations of serum vitamin B_{12} concentrations were made using *Euglena gracilis, var. bacillarus* as the test

organism. All of the serum vitamin B_{12} levels in patients with liver disease, with the exception of those with biliary cirrhosis, were significantly elevated over the normal control levels. The most abnormal levels occurred at a time when the patient was most acutely ill. The levels tended to return to normal levels as the patients improved clinically.

The capacity of serum to combine with vitamin B_{12} in *vitro* was also studied. In general, it was found that if adequate amounts of free vitamin B_{12} were present in the serum, very little additional vitamin B_{12} could be found, regardless of the amount added. There was no difference between normal individuals and those with liver disease as regards binding.

This is a clear-cut study and the only question would be if any acutely ill person might show the same pattern as the patients with liver disease. It might be well to include this type of patient in the control group.

K. R. CRISPELL

Malignant Growth in the Liver and Serum-Vitamin-B_{12} Levels. N. Grossowicz, A. Hochman, J. Aronovitch, G. Izak, and M. Rachmilewitz. *Lancet* 1:1116, 1957.

It has been previously shown that high serum vitamin-B_{12} levels accompany acute liver disease; the assumption has been that the injured liver cells released the vitamin to the blood stream. Serum vitamin-B_{12} levels were estimated microbiologically using *E. coli* in 18 cases of carcinoma with liver involvement and 19 cases of carcinoma without metastases in the liver. In sixteen of the cases where the liver was involved the serum vitamin-B_{12} was markedly raised to from 640 to 20,000 $\mu\mu$g per ml. One of the other two cases with a serum-vitamin-B_{12} level of 470 $\mu\mu$g had a localized hepatoma; the other (with a level of 500 $\mu\mu$g) had carcinomatosis from an unknown primary growth. In the 19 cases of carcinoma without liver involvement the serum vitamin-B_{12} levels were normal (100–550 $\mu\mu$g/ml). These findings seem to offer a diagnostic tool of considerable value.

F. E. HYTTEN

The utilization of amino acids in hepatic disease is impaired as shown by elevated serum levels. Protein biosynthesis is reduced in muscle as well as liver tissue in patients with severe hepatic dysfunction.

Post-prandial Blood Amino Acid Patterns in Patients with Hepatic Anorexia. S. M. Mellinkoff, M. Frankland, M. Greipel, H. N. Shibata, and W. J. Dixon. *Gastroenterology* 32:592, 1957.

There is evidence that nausea and anorexia appear as blood amino acid levels rise following the feeding of proteins or the administration of amino acids by infusion. The authors have previously attempted to correlate the anorexia of patients with chronic liver disorders with *total* amino acid levels but found that the total levels were normal. In this report they have extended their study to determine if there were qualitative differences in blood amino acid patterns in patients with hepatic anorexia.

Fasting and post-prandial blood amino acid patterns were appraised visually and analyzed by densitometric measurement in 25 normal controls, 21 patients with hepatic anorexia, and 9 patients with liver disease and a good appetite. The authors were of the opinion that by inspection of the chromatograms they could predict whether or not the patient had hepatic anorexia. No attempt was made to analyze all possible changes. Some of the criteria employed for analyzing the chromatograms were the following: high fasting cystine for liver disease with poor appetite; comparatively high post-prandial leucine and valine for liver disease with good appetite; and comparatively low post-prandial alpha-alanine for normal controls.

The authors conclude that there are changes in the amino acid pattern in patients with hepatic anorexia which differ from those with chronic liver disease and good appetites, but do not claim that there is a causal relationship between appetite and amino acid patterns.

J. B. HAMMOND

The following investigation provides a sound basis for the use of some oral antibiotics such as neomycin, in patients with severe liver insufficiency. Removal of the products of bacterial metabolism requiring detoxication by the liver has been suggested as the basis for the salutary effects occasionally observed clinically with this therapy.

The Role of Intestinal Bacteria in the Development of Dietary Cirrhosis in Rats. A. M. Rutenberg, E. Sonnenblick, I. Coven, H. A. Aprahamian, L. Reiner, and J. Fine. *J. Exper. Med.* 106:1, 1957.

The administration of antibiotics delays the development of dietary cirrhosis in rats. This effect is lost after the bacterial intestinal flora becomes resistant to antibiotics. Rats fed a necrogenic diet and maintained in a germ free environment do not develop hepatic necrosis. In order to test the role of intestinal bacteria in the development of dietary cirrhosis, the following experiments were carried out: Male rats of the Wistar strain weighing about 90 g were fed *ad libitum* a diet consisting of choline-free peanut meal and casein supplemented with lard and adequate amounts of vitamins and minerals. The animals were divided into the following groups: (1) choline-free without antibiotics, (2) choline-free plus choline supplement, (3), (4), and (5) choline-free plus 25 mg/day or 50 mg/day tetracyclin or 15 mg/day neomycin, (6) choline-1,000 units/day bacitracin and 15 mg/day neomycin, (7) choline-free plus 1,000 units/day bacitracin and 30,000 units/day polymyxin B.

The animals were observed up to 750 days. Bacteriologic studies of blood and tissues and microscopic examinations of the liver were carried out. Of the rats fed the choline-free diet without antibiotics 80 per cent developed cirrhosis; of rats fed the choline-free diet plus absorbable antibiotics 73 per cent, but of those

kept on the choline-free diet supplemented by poorly or nonabsorbable antibiotics only about 20 per cent developed cirrhosis. Therefore, liver cirrhosis in rats is thought to be caused by intestinal bacteria and not by choline-deficiency. On the other hand, the fatty infiltration of the liver caused by choline-deficiency was not prevented by antibiotic therapy.

M. SILBERBERG

The hepatic-repair dietary program does not emphasize fat restriction. A nutritious high-protein diet is required unless intolerance to nitrogen is manifested by encephalopathic symptoms. Thioctic acid therapy is still under investigation and may hold promise for the future.

Diet and Nutritional Aids in Liver Disease. V. M. Sborov. *Am. J. Digest. Dis.* N.S. 3:94, 1958.

In a compact but comprehensive review of the dietary treatment of liver disease, the author points out that certain hepatic diseases, once induced by nutritional deficiency, may be irreversible and correction of the dietary defect may result in no improvement in the disease. He suggests that in some conditions, dietary therapy may cause slight improvement not detected by ordinary clinical means.

For the patient with acute viral hepatitis, an attractive, well-balanced diet which supplies 3,000 calories or more and includes 100 to 120 g of protein, 120 to 175 g of fat, and 400 to 500 g of carbohydrate is advised. No adequate rationale for fat restriction has been found from various studies. During the acute phase, when anorexia is a problem, the patient should be encouraged to eat and preferably should not be tube-fed. Vitamin B_{12} may be of value but methionine and choline have been found ineffective. Despite reports to the contrary, the author feels that alcohol is contraindicated for six months after recovery. If hepatic coma supervenes from either virus hepatitis or other acute forms of liver damage, a protein-free diet is indicated to minimize ammonia intoxication.

In chronic virus hepatitis associated with intermittent anorexia and nausea, the use of intravenous glucose, amino acids, and albumin may be necessary to maintain adequate nutrition. Albumin solution should be infused slowly to avoid hypervolemia and ensuing pulmonary edema or bleeding from esophageal varices.

Simple fatty liver, which has not yet progressed to cirrhosis, has an excellent prognosis provided alcohol is withheld and a balanced diet maintained consistently. Lipotropic agents have not been of value in this condition.

In Laennec's cirrhosis not associated with alcohol excess, the prognosis for those who adhere to an adequate, nutritious diet is better than in similar patients who are chronic alcoholics.

In patients with edema and ascites associated with cirrhosis of the liver, sodium restriction to 200 mg/day, which is approximately the amount excreted, is usually effective. A satisfactory program involves the administration of 10 to 20 mg of Prednisone daily, with 250 mg/day of Diamox, or an intramuscular mercurial diuretic. Potassium salts usually are prescribed unless renal disease or oliguria are present.

J. B. HAMMOND

Therapy of Fatty Liver and Fatty Cirrhosis with Thioctic Acid. F. Rausch. *Ärztl. Forsch.* 16: 79, 1958.

The author reports on the therapeutic results of thioctic acid in cases of human fatty liver and fatty cirrhosis in 11 patients. Liver biopsies demonstrated that the thioctic acid exerts a hepatolipotropic effect in man similar to that previously observed in animals. Apart from its effect in cases of hepatic coma, this chemically, clearly defined substance, with the biologic properties of a catalyzer, can be advocated as a therapeutic agent in cases of fatty degeneration of the liver.

AUTHOR

Changes in the Composition of Cirrhotic Livers of Rats Following the Feeding of Extra Protein or Lipotropic Factors. W. G. B. Casselman and R. J. Young. *Proc. Canad. Fed. Biol. Soc.* 1:11, 1958.

Rats fed a 9 per cent protein-hypolipotropic diet for 200 days developed cirrhosis of the liver. For an additional 200 days groups of such cirrhotic rats were given diets supplemented with choline, betaine, or methionine and containing 9 per cent, 20 per cent, or 40 per cent protein. In the liver, total lipids, dry fat-free residue, ribonucleic acid, desoxyribonucleic acid, protein and hydroxyproline contents were determined. The results were similar in all groups and consisted of decrease of total lipids, increase in ribonucleic acid and protein, but no change in total collagen as compared with findings in the cirrhotic controls which had not received dietary supplements. Therefore, the protein and vitamin-enriched diet apparently restored the usual composition of the liver cells but failed to affect increase in the amount of collagen.

M. SILBERBERG

The Spleen in Ethionine-Induced Cirrhosis. Its Role in γ-Globulin Elevation. G. Kent, H. Popper, A. Dubin, and C. Bruce. *A.M.A. Arch. Path.* 64:398, 1957.

Female Sprague-Dawley rats were fed a synthetic hepatotoxic diet supplemented with 0.3 per cent or 0.5 per cent ethionine. After a certain period, when the rats were expected to have liver damage, some animals were allowed alternating periods of feeding on a stock diet. One group of rats was splenectomized and fed correspondingly. The rats were observed for periods varying from 8 to 729 days. Values of γ-globulin levels in the blood were determined during different periods of the experiments, and at necropsy liver, spleen, bone marrow, and lymph nodes were examined microscopically.

The liver showed changes developing in the following order: (1) central lobular necrosis, (2) diffuse hepatitis, (3) septal cirrhosis, and (4) coarse nodular cirrhosis. The spleen showed increasing enlargement up to ten times the normal. The microscopic changes consisted of (1) hyperplasia of pulp cells, (2) engorgement of sinusoids with fibrosis of pulp; (3) follicular fibrosis; (4) perifollicular periarterial hemorrhages; and (5) extramedullary hemopoiesis. The abdominal lymph nodes were markedly enlarged and contained an increased number of pyronine staining cells. Essentially the bone marrow was unchanged. The lesions were most accentuated in the cirrhotic stage and if the high dose of ethionine was given. Parallel to the severity of the liver changes was a rise in the γ-globulins. After feeding the stock diet the hyperplasia in the spleen regressed, and the γ-globulin values returned to normal while the liver cirrhosis persisted. Splenectomy had no effect on the observed changes.

M. SILBERBERG

The so-called "bush-tea" cirrhosis observed in Jamaican patients is a recently described entity of considerable interest to nutritionists.

Plants as Aetiological Factor in Veno-occlusive Disease of the Liver. G. Bras, D. M. Berry, and P. György. *Lancet* 1:960, 1957.

A particular form of infantile hepatic cirrhosis has been reported from Jamaica in which the characteristic lesion is venous occlusion. It has been suspected that the condition is of toxic origin and the local habit of making decoctions of various leaves for medicinal purposes has been blamed. In experiments with calves and horses feeding off the plants *Crotalaria* and *Senecio* has in some cases produced the characteristic veno-occlusive lesions and it is suggested that the use of these plants for "bush tea" may be the cause of the condition in infants. F. E. HYTTEN

Veno-occlusive Disease of the Liver. K. L. Stuart and G. Bras. *Quart. J. Med.* 26:291, 1957.

Clinical and histologic findings from 84 Jamaican patients are reported, of whom 73 were followed up for six months to five years. The disease has three overlapping stages; an acute phase, particularly in children, with hepatomegaly and ascites, a subacute stage in which hepatomegaly, often symptomless, is the prominent finding; and a chronic stage, which may be indistinguishable from cirrhosis of other etiologies but which may progress rapidly. Recovery occurred in 48 per cent of patients and was possible from both the acute and subacute stages. Prognosis was worst in adults and infants under a year of age; it was related to liver damage as reflected by serum albumin concentrations, jaundice being relatively rare.

Serial liver biopsies suggested the disease progressed from an acute, widespread occlusion of the smaller hepatic vein branches, with pressure necrosis of liver cells, to a nonportal cirrhosis around the central veins. Hepatomegaly and ascites were attributed mainly to obstruction of the hepatic venous outflow tract. Malnutrition was thought to play an important, but subsidiary, part in the disease although fatty change was common in affected infants, thus, perhaps, determining their poorer prognosis. It was suggested further that malnutrition predisposed the liver to injury from local plant toxins (possibly *Senecio* or *Crotalaria retusa* which are used in "bush teas") and that these were primarily responsible for the disease. W. H. J. SUMMERSKILL

The effectiveness of the commonly used serum tests for differentiating types of jaundice varies. The cephalum flocculation test gave best results, but the alkaline phosphatase and thymol turbidity tests are recommended for substantiation.

Discrimination Between Obstructive and Hepato-Cellular Jaundice by Means of the Commonly Used Serum Tests. E. Hill and L. Zieve. *Am. J. Clin. Path.* 27:6, 1957.

Comparative effectiveness, separately and in combination, of the cephalin-cholesterol flocculation test, thymol turbidity test, zinc sulfate turbidity, alkaline phosphatase (King-Armstrong), and serum cholesterol determination was studied in 49 patients with obstructive jaundice, and in another 49 patients with hepatocellular jaundice (24 of viral origin). Patients were paired individually, between the two groups, according to the degree of bilirubinemia of their sera.

The cephalin flocculation test was considered the best for differentiation between the two types of jaundice. Statistical analysis of the data employed a discrimination ratio (a ratio of the total variation observed in both groups together, which is due to average differences between the groups). Thymol turbidity tests were considered two-thirds as effective, the alkaline phosphatase and zinc turbidity tests were only half as effective, with the serum cholesterol test being only two-fifths as effective for differentiation of the types of jaundice.

Although the cephalin-cholesterol flocculation test is considered the most valid, the authors recommend the alkaline phosphatase and thymol turbidity tests for substantiation of results. Their recommendation is justified because turbidity or flocculation tests determine alterations of the proportions of serum proteins. Furthermore, such tests do not indicate directly a change in the liver, or its physiology, but merely give a clue to ratios of large and small protein molecules. Although many workers have attempted to assess liver function tests, data have not substantiated that any one is better than all of the others. Most of the data do point to the effectiveness of the cephalin-cholesterol flocculation test as a guide for the separation of the cases which according to other tests may give evidence

of both liver cell damage *and* interference of the flow of bile. E. COHEN

FAT METABOLISM

Mobilization of fat from tissue depots is enhanced by certain hormones such as cortisone, somatotropin, and thyroid hormone. The lipid-mobilizing factor described by Seifter and Baeder appears to be a hormone active in the fasted individual in elevating all fractions of serum lipid.

Metabolic Studies in Patients Receiving Lipid Mobilizer Hormone. C. J. D. Zarafonetis, G. M. Miller, J. Seifter, D. Baeder, R. M. Myerson, and W. A. Steiger. *Am. J. M. Sc.* 234: 493, 1957.

Lipid-mobilizing hormone (LM) is extracted from plasma of cortisone-treated animals. It is capable of producing hyperlipemia and of inhibiting delactescence of serum of the heparin-clearing factor upon injection into animals or man. This hormone has been recovered from the posterior pituitary of hogs. The administration of LM daily for two weeks to four patients maintained on a low-fat diet resulted in a remarkable elevation of plasma cholesterol, fatty acids, and lipid phosphorus. There was a distinct increase in fecal and urinary cholesterol excretion. Two patients maintained on high-fat intakes exhibited no significant change in serum lipid values when given LM. There were no significant side effects except in one patient, also receiving Isoniazid, who developed mental confusion and signs of hepatitis during the second week of LM administration. The response to LM appears to depend upon the metabolic conditions extant at the time of its injection. A diet low in fat permits marked hyperlipemia after LM administration, while a high fat intake blocks this response. C. R. SHUMAN

On the Fat-Mobilising Activity of Human Urine. T. M. Chalmers, A. Kekwick, and G. L. S. Pawan. *Lancet* 1: 866, 1958.

A substance or complex, which has been partially purified by chromatography, appears in the urine of healthy persons who have been fasting. When the substance is injected into mice it mobilizes depot fat, causes deposition of fat in the liver, increases the metabolic turnover of fat, and causes weight loss without loss of appetite. The substance, called here "fat mobilising substance" cannot be detected in urine from the same people when they are taking a normal diet.

F. E. HYTTEN

The Effect of Growth Hormone on the Uptake of Neutral Fat by the Liver In Vitro. M. Héimberg, H. C. Meng, and D. Bradley. *Endocrinology* 62: 682, 1958.

The influence of growth hormone on the uptake of neutral fat by the liver has been investigated. This study is an extension of earlier observations which showed that crude anterior pituitary extracts markedly increased hepatic lipid uptake. This investigation showed that administration of growth hormone to the intact rat 3 to 8 hours before removal of the liver resulted in an increased uptake of neutral fat when the liver was perfused in vitro. It was also shown that addition of growth hormone directly to the fluid perfusing the liver also increased lipid uptake. This observation indicates that growth hormone in addition to the other hormones such as ACTH have an effect on the metabolism of fat. A. B. EISENSTEIN

Both insulin and glucose have been shown to reduce nonesterified fatty acids, the most actively metabolized lipid fraction. Perhaps the utilization of carbohydrate inhibits the release of fatty acids while the rate of utilization of fatty acids continues, thus lowering serum levels.

Action of Insulin on Release of Fatty Acids From Tissue Stores. E. L. Bierman, I. L. Schwartz, and V. P. Dole. *Am. J. Physiol.* 191: 359, 1957.

The mechanism by which carbohydrate utilization reduces the concentration of nonesterified fatty acids (NEFA) in plasma was studied by comparing the clearance of C^{14}-labeled palmitic acid before and after the administration of insulin. The rate of disappearance from blood of a single injection of C^{14}-labeled palmitic acid was identical before and after an intravenous injection of insulin (0.1 μ/kg) although the expected significant fall in total NEFA concentration occurred. When steady concentration of labeled NEFA was maintained by a constant infusion, the administration of insulin produced a significant increase in specific activity. It is, therefore, concluded that insulin decreases the release of fatty acids from tissue stores but does not accelerate their removal from blood. AUTHORS

The Effect of Adrenergic Blocking Agents (Including Chlorpromazine) on Serum Lipid Levels of Patients with Disorders of Fat Metabolism. L. E. Hollister. *J. Chron. Dis.* 6: 234, 1957.

The author presents studies on the effect of adrenergic blocking agents, including chlorpromazine, on serum lipid levels of patients with disorders of fat metabolism. The basis for this study was that subcutaneous administration of large doses of epinephrine in oil to dogs produced a rise in plasma phospholipid, cholesterol, and esterified fatty acids. This rise was blocked by administration of adrenergic blocking agents. Chlorpromazine is thought by some investigators to be a moderately active adrenergic blocking agent.

The effect of four drugs on serum cholesterol, phospholipid, and total lipids was studied in 13 patients with a variety of disorders of fat metabolism. Five out of 12 patients treated with chlorpromazine obtained significant reductions in two or more lipid fractions. Dibenzyline, hydergine, and Regitine gave inconstant

results. "At present these observations are of more physiologic interest than therapeutic value."

K. R. CRISPELL

Effects of Carbohydrate Feeding on Serum Lipids and Lipoproteins in the Rat. J. Bragdon, R. Havel, and R. Gordon, Jr. *Am. J. Physiol.* 189: 63, 1957.

Fasting in the rat is accompanied by an increase in serum cholesterol concentration, reflecting an increase in high-density lipoproteins. The feeding of carbohydrate results in decreases in both low- and high-density lipoproteins, the former occurring acutely, and the latter occurring more slowly. In the fasting rate the injection of protamine, an antiheparin agent, produces an increase in all serum lipids, but the increase occurs in the low-density lipoproteins. In the rat fed carbohydrate this lipemia-inducing effect of protamine is practically abolished. The feeding of carbohydrate has no effect, however, on the rate of clearance of intravenously administered chylomicrons. These phenomena are discussed in relation to current theories of lipid transport.

AUTHORS

The transport of fat and cholesterol derived from hepatic lipogenesis results in an elevation of serum lipid fractions. The relation of this process to weight gain is an important matter.

Weight Gain from Simple Overeating: II. Serum Lipids and Blood Volume. J. T. Anderson, A. Lawler, and A. Keys. *J. Clin. Invest.* 36: 81, 1957.

This study was an attempt to produce obesity experimentally in man by simple overfeeding, without a substantial change in physical activity, using for this purpose a diet in which the calorie increase is provided one-third by fat and two-thirds by carbohydrate. This would resemble a situation in which no significant change in the diet except for the more liberal use of bread and potatoes, together with such fat as is ordinarily eaten with these, may have taken place.

Following an initial standardization period of six weeks, overfeeding of 20 schizophrenic male patients under controlled conditions for 20 weeks resulted in an average body weight gain of 23 lb. Gains of from 43 to 49 lb were obtained in four men who were most successfully persuaded to eat more than they had selected. The average calorie increase ranged from 8 to 39 per cent.

The average total serum cholesterol concentration rose 20 mg per 100 ml during the first five weeks of overeating and then remained substantially constant during the next 15 weeks, although the weight gain continued at essentially the same rate as it had during the first five weeks. During the tenth to twentieth weeks of overeating, although the serum cholesterol level remained constant, the concentration of the S_f 12 to 20 lipoprotein fraction tended to increase. A moderate increase in the circulating plasma and blood volumes occurred during the first weeks of overeating.

The authors suggest that, other things being equal, the serum cholesterol concentration is determined by the fat transport load imposed on the blood per unit of circulation. At calorie equilibrium, this is determined by the proportion of calories present as fats, and this relationship is not altered by increasing the energy level of intake if calorie equilibrium is maintained by increased exercise, which normally also involves a proportion of increase in circulatory rate. If the calorie balance is positive, the fat transport load is obviously increased.

If fat storage is taking place, that portion of the fat synthesized in the liver from carbohydrate also adds to the transport load so that some serum cholesterol levels rise during the active phase of gaining weight, even on a reduced fat diet. If the calorie excess and the weight gain continue steadily, there is no further increase in the fat transport load and the serum cholesterol levels should remain constant at this newly raised level.

Furthermore, if calorie equilibrium is now achieved and obesity is in a steady state, the serum cholesterol level would approximate that characteristically associated with the proportion of fat in the diet at equilibrium. This is an interesting hypothesis and deserves careful consideration.

S. O. W.

The formation of molecules of fatty acid from acetate is believed to require a source of hydrogen ions derived from TPNH together with energy provided by utilization of glucose. Apparently adrenal steroid hormones are not required so long as an adequate supply of carbohydrate is provided from dietary sources.

Effect of Food Administration on Lipogenesis in Normal and Adrenalectomized Rats. C. Cohn and D. Joseph. *Am. J. Physiol.* 189: 68, 1957.

Normal and adrenalectomized rats were either force-fed or allowed to eat ad libitum a high carbohydrate diet for three to four weeks. The food intake of the force-fed groups was adjusted so that the rate of weight gain of the normal animals was equal to that of normal rats eating ad libitum. During the last two days of life, C^{14}-labeled sodium acetate was included in the diet and its rate of incorporation into body fat was measured. The results reveal that under these conditions, the force-fed adrenalectomized rat gained weight at the same rate as normal force-fed controls. Furthermore, the adrenal-steroid-deficient animal fed in this manner contained amounts of body fat and incorporated C^{14} into the lipid moiety in quantities that did not differ from those of force-fed normal rats. By contrast, the adrenalectomized rat eating ad libitum gained less weight, contained less body fat but incorporated more C^{14} into his fat fraction than normal controls. The authors conclude that in the well-nourished state no deviation from normal in lipogenesis exists in the animal lacking his adrenal glands.

AUTHORS

Despite advances in biochemical knowledge of fat metabolism, there is little information available concerning the defects involved in the pathogenesis of idiopathic hyperlipemia. Present management of these cases requires the marked curtailment of fat intake.

Idiopathic Hyperlipaemia. K. S. Holt. *Arch. Dis. Childhood* 32: 142, 1957.

Fewer than 50 cases of this syndrome have been described in all age groups. This report documents an additional instance in a seven-year-old boy who was admitted to the hospital for acute tonsillitis. He had apparently completely recovered from an attack of infectious hepatitis seven months previously. During the previous two years, he had had six attacks of abdominal pain and fever which lasted only 24 hours. Examination revealed smooth, firm, nontender enlargement of both liver and spleen. Lipemia retinalis was present. No xanthomata were found. Total serum lipids were 4 g/100 ml. Foam cells formed 1.6 per cent of the bone marrow. The serum proteins were albumin 3.2 g and globulin 4.0 g/100 ml. Radiography showed no intra-abdominal calcification and the subcutaneous tissues were of normal thickness. His parents and two sisters did not show clinical evidence of hyperlipemia, the serum lipids ranging from 360 to 670 mg/100 ml. The hyperlipemia persisted while patient took a normal diet, the highest level reached being 7.7 g/100 ml. Within 48 hours after taking a diet containing less than 5 g of fat a day, the retinal vessels appeared normal and one day later, the serum lipids had fallen to 1 g/100 ml. No change in size of liver or spleen occurred while on such a diet for one week, nor did change occur after one year on a diet containing 15 g of fat per day.

Following administration of potassium iodide to block uptake by thyroid, radio-iodinated (I^{131}) olive oil was incorporated into butterfat and fed in amount of 5 μc. The radioactive iodine which appeared in the urine indicated the quantity of fat which had been metabolized. Three tests were performed while the patient was on a diet containing 45 g, 5 g, and 15 g of fat daily. Results were compared to those obtained by Sammons and Pover (Third International Congress of Biochemistry, Brussels, 1955). No evidence of delay in metabolism of the labeled fat was found. Therapy with low-fat diet was most beneficial. T. C. Panos

Serum Lipids in Adult Twins. R. H. Osborne and D. Adlersberg. *Science* 127: 1294, 1958.

Twins, ranging in age from 18 to 55 years and residing in New York City, were studied. Total serum cholesterol, esters, and phospholipids were obtained for 82 of the twin pairs found to be in good general health. Twin zygosity was established by serologic and morphologic comparison.

The intrapair variances of monozygotic twins did not differ significantly from those of dizygotic twins of the same sex. However, the dizygotic variance was the larger. Furthermore, in both mono- and dizygotic twins the variances of pairs living apart were larger than for pairs living together. Thus, the closest relationship of serum lipids was found in monozygotic twins living together. At the other extreme were dizygotic twins living apart. The authors conclude that this analysis demonstrates that variations in lipid levels results from both genetic and environmental influences. In the authors' words, "It may be assumed that genetic factors will not be equally important in all environments and that environmental tolerances of one population will not equal those of another." S. O. W.

Oxidation of fatty acids to CO_2 is depressed in glycogen-depleted animals; however, the rate of formation of keto-acids and acetone is found generally to be increased. The reduction in fatty acid oxidation in the absence of available glucose may be related to a decrease in oxaloacetate derived from this source.

Role of Carbohydrate Metabolism in Promoting Fatty Acid Oxidation. E. J. Masoro and J. M. Felts. *J. Biol. Chem.* 231: 347, 1958.

The oxidation of carboxy-labeled fatty acids to $C^{14}O_2$ was studied using rat liver slices from glycogen-depleted animals which were fasted for 24 hours while exposed to cold. Conversions of labeled butyrate, pentanoate, hexanoate, heptanoate, octanoate, and decanoate to $C^{14}O_2$ by liver slices from cold-fasted rats were depressed. Addition of 0.02M glucose partially restored the rate of oxidative decarboxylation by slices from cold-fasted rats, but had no effect on samples from control animals. The formation of $C^{14}O_2$ from the labeled long-chain acids, laurate, myristate, palmitate, stearate, and oleate by liver slices from cold-fasted rats was not depressed. However, the addition of 0.2M glucose stimulated the rate of oxidation, especially of the C_{16} and C_{18} acids. On the other hand, glucose depressed the oxidation of C_{16} and C_{18} acids by control slices. Although both lactate and succinate were found to traverse the Krebs cycle at about the same rates, lactate stimulated the metabolism of acetate-1-C^{14} and palmitate-1-C^{14} but succinate had no effect. It appeared that glucose or some metabolizing carbohydrate is needed for optimal oxidation of fatty acids, but this effect does not involve the "sparking" action of the Krebs cycle. In contrast, glucose depressed long-chain fatty acid oxidation, thereby exerting a sparing action in control "carbohydrate adequate" mixtures.

M. K. Horwitt

The increased retention of nitrogen associated with feeding of sunflower oil, a source of unsaturated fatty acid, is of considerable interest.

Dietary Fats of Different Varieties and Their Effect on the Digestion of Fat and Retention of Nitrogen. M. N. Markova. *Voprosy Pitaniia* (Moscow) 16: 15, 1957.

The subjects of study were the digestibility of sunflower oil, lard, combined fat, and synthetic fat and

their effect on the retention of nitrogen. Experiments were made on 60 male white rats weighing from 170 to 200 g.

The following was established: (1) in the experiment on rats the digestibility of all fats was equal; (2) the investigated fats affected the retention of nitrogen, absorption was greatest when the food included sunflower oil, less with lard and combined fat, and considerably lower with synthetic fat; (3) nitrogen retention was affected by the length of time during which the animal was fed with the same fat. AUTHOR

Role of Butterfat in Nutrition and in Atherosclerosis: A Review. F. A. Kummerow. *J. Dairy Science* 40: 1350, 1957.

In addition to a review of the literature on the subject (44 references), the author reports the results of a number of his observations and experiments. His researches have demonstrated in animal studies that a high-fat diet increases serum cholesterol only when the level of dietary protein is inadequate. He concludes that the ratio of dietary protein to dietary fat may be more important to serum cholesterol levels than soft or hard fats.

The author warns against placing too much emphasis on serum cholesterol as a criterion of normal metabolism; certain experimental conditions may merely cause a shift of cholesterol from blood to liver or carcass. Researches in this field should provide for complete balance studies on total as well as serum cholesterol. The application of excessive heat in presence of air, such as might occur in food preparation, does less damage to the nutritive value of butterfat than to such fats as corn, cottonseed, olive, and soybean oils that contain substantial quantities of triunsaturated glycerides, which under such conditions readily undergoes polymerization and oxidation. F. E. RICE

The following group of summaries presents the various viewpoints on the effect of ingestion of fats upon the coagulability of blood. This has been a highly controversial subject, with some investigators demonstrating no effect while others have found significant increases in blood clotting following a high fat intake.

Dietary Restriction and Coagulability of the Blood in Ischaemic Heart Disease. L. McDonald and M. Edgill. *Lancet* 1: 996, 1958.

The following tests were performed on 34 subjects with ischemic heart disease before and after they had been maintained on a rice-fruit diet for four to five weeks: thromboplastin-generation test, platelet count, estimation of platelet stickiness, fibrinogen estimation, and prothrombin (Stypven) time.

The results are presented in considerable statistical detail, although the numbers of subjects left for comparison after various exclusions are few. There was a decrease in platelet stickiness after the diet together with a reduced level of blood cholesterol. All subjects lost weight on it. No difference was found in thromboplastin generation, fibrinogen estimation, and prothrombin time. F. E. HYTTEN

Fat Ingestion, Blood Coagulation and Atherosclerosis. J. R. O'Brien. *Am. J. M. Sc.* 234: 373, 1957.

The possible association between the ingestion of fats and coronary artery disease may lie in part in the increased coagulability of blood after a fatty meal inducing the formation of mural clots which develop into an atheromatous plaque. The lack of information concerning the physiochemical properties of the various lipids found in the blood after fat ingestion adds to the complexity of the problem. A variety of methods have been employed to demonstrate changes in the coagulability of blood after fat ingestion. The author has studied the effect of such phospholipids as phosphatidylethanolamine upon the Stypven time, a measure of the clotting time of recalcified plasma to which Russell viper venom has been added. Shortening of the clotting time has been demonstrated with this phospholipid. The ingestion of fatty meals containing substances in which the active phospholipids are found causes acceleration of the plasma clotting time. The influence of the degree of saturation of fats upon serum cholesterol and beta lipoprotein levels is discussed; coronary thrombosis is associated with high levels of these lipid components according to some authorities. Of the lipid fractions studied, the phospholipids exert the greatest influence upon clotting mechanism. A decrease in the normal fibrinolytic property of blood is markedly decreased in the presence of lipemia, an effect made more prominent by the feeding of saturated fats. It is suggested that the phospholipids participate in the phenomenon of atherosclerosis because of their role in the coagulation of blood. C. R. SHUMAN

Anti-lipaemic Agent Without Anti-coagulant Action. E. M. M. Besterman and J. Evans. *Brit. M. J.* 1: 310, 1957.

Heparin has been shown to reduce the abnormal lipemia associated with atheroma, but there is no convincing evidence that prolonged use of the drug would cause a regression of the disease. Such a course would in any case be dangerous because of the anticoagulant properties.

A polysaccharide—laminarin—derived from seaweed can be sulfated in varying degrees, the highly sulfated compounds having strong anticoagulant powers, the ones with few sulfate groups having little or no effect on blood clotting.

Two preparations of laminarin with a low sulfate content, *M* and *N*, were tested in nine patients with ischemic heart disease and compared to the action of heparin in the same patients. The effects were variable; but, in general, lipemic serum was cleared and the distribution of lipoproteins was altered as it is with heparin. There was no anticoagulant ef-

fect and no toxic or local reactions occurred. The effect could not be duplicated in vitro.

Laminarin "would appear to be a suitable agent to assess the effect of 'lipaemic-clearing' substances on the course of atherosclerosis in man." F. E. HYTTEN

Blood Coagulation Before and After a Fatty Meal. J. R. O'Brien. *Lancet* 1: 410, 1958.

The following blood coagulation tests were performed for 21 men aged 40 to 60 with a previous coronary thrombosis and 21 healthy men of the same age: whole-blood clotting time in siliconized tubes; whole-blood clotting time in ordinary glass tubes; the same with powdered glass added; "Stypven time" of citrated blood; Stypven time of plasma and diluted plasma and the heptane-Stypven ("accelerated") clotting time.

The tests were done before and four hours after a standard fat meal. Both groups behaved similarly; both showed postprandial changes in all tests but this was most marked in the powdered-glass test. "These findings lend no support to the suggestion that postprandial hypercoagulability is responsible for the alleged connection between the fat content of the diet and coronary thrombosis." F. E. HYTTEN

Production by Various Fats of Myocardial Necroses in Humorally Conditioned Rats. H. Selye. *Proc. Soc. Exper. Biol. & Med.* 98: 61, 1958.

Female rats of the Sprague-Dawley strains weighing about 100 g received, for a period of six days, two daily injections of 100 μg of 2α-methyl-9α-chlorocortisol and Na_2HPO_4 by stomach tube. The animals were kept on diets supplemented with 0.5 or 1 ml. of corn oil, peanut oil, pork fat, chicken fat, or paraffin oil. Treatment with cortisol Na_2HPO_4 caused myocardial necrosis and nephrocalcinosis. The severity of these lesions was markedly intensified by feeding the diets enriched with plant or animal fats, but not by those enriched with mineral oil. M. SILBERBERG

UNSATURATED FATTY ACIDS

Characteristic of the medical literature dealing with the problem of dietary fat, cholesterol, and arteriosclerosis, great enthusiasm has developed for the most recent contributions on the subject of unsaturated fatty acids as a means of lowering serum cholesterol and possibly reducing the liability to atherosclerotic disease. It seems likely that the advances in this field represent a tangible means for achieving the desired ends without drastic inconvenience to the patient. However, carefully planned and objective nutritional studies are needed to evaluate the efficacy and practicality of increasing sources of dietary unsaturated fatty acid.

Essential Fatty Acids and Atherosclerosis. G. V. Mann. *Arch. Int. Med.* 100: 77, 1957.

"A critique of the present knowledge" concerned with the relation of dietary fat to atherosclerosis is presented by an expert in the field. He has reviewed, critically analyzed, and judged most of the work relating to this important problem. Answers are not forthcoming—only avenues of approach. A great many of the discordant observations in animals may well be due to species differences and unnoticed dietary vagaries.

Data in humans are rapidly accumulating which show, rather convincingly, that the degree of unsaturation of dietary fat is the crucial determinant for serum cholesterol reduction, not the amount of cholesterol or fat in the diet. That this is an effective means of preventing, much less reducing, atherosclerosis, of course is the question that remains to be answered. Furthermore, the relationship of dietary fat to coronary artery thrombosis is yet another problem, perhaps better related to lipemia and its effect on blood coagulation.

Certainly, there are many unanswered questions which Dr. Mann raises. Anyone interested in this problem should read the paper. At the present time there appears to be enough presumptive evidence to allow the practicing physician to prescribe a diet containing a reduced amount of fat as the one positive approach to the problem. What proportion of the ingested fat should be unsaturated is at present an unanswered question; however it would seem reasonable at the moment to advise the use of as much vegetable fat as possible. J. F. MUELLER

Low-fat diets have been found less effective in reducing serum cholesterol than have diets high in unsaturated fats. This suggests that unsaturated fatty acid-cholesterol esters are utilized more efficiently and rapidly by tissues resulting in a lowering of serum cholesterol.

Essential Fatty Acids Lipid Metabolism and Atherosclerosis. L. W. Kinsell, G. D. Michaels, R. W. Friskey, and S. Splitter. *Lancet* 1: 334, 1958.

This report contains a condensation of much recent work by this team of workers and deserves to be read in the original. It is presented as a series of questions and the answers are illustrated by metabolic data from a number of subjects.

It is established that polyunsaturated fatty acids *per se* lower the plasma cholesterol level as effectively as do natural fats containing the same amounts of these acids. There is tentative evidence that linoleic acid is an "essential" fatty acid in man. Changes in plasma lipid levels associated with the ingestion of fats containing linoleic acid probably have a beneficial effect on atherosclerosis; nothing is known of the mode of action of fatty acids in lowering the plasma cholesterol level.

The inclusion in the diet of natural fats containing 40 to 80 per cent of linoleic acid lowers the plasma lipid level but the extent of the lowering depends on the composition of the diet and the clinical status of the subject. Diets high in essential fatty acids are more effective in lowering plasma lipid levels than a very low-fat diet. Arachidonic acid may be much more potent

as a hypocholesteremic agent than linoleic acid. F. E. HYTTEN

The Effect on Serum-Cholesterol of Diets Containing Different Fats. H. Malmros and G. Wigand. *Lancet* 2: 1, 1957.

This paper is another minor variation on what must be the most popular theme in nutrition. A number of normal subjects and a number of persons with familial essential hypercholesteremia were investigated. Each subject received a variety of fats at the rate of about 150 g daily, supplying about 40 per cent of the calories. Many of the vegetable fats were made into palatable milk, cheese and ice-cream substitutes and the diets well tolerated for long periods; a single type of diet was usually maintained for a month.

The results, which are no longer unexpected, are given in detail: of the vegetable fats, corn oil and sunflower-seed oil had a markedly depressing effect on serum cholesterol, rapeseed oil a less marked effect, and olive oil a slight effect. Coconut oil, hydrogenated or not, has no effect. Of the animal fats, milk enhanced serum cholesterol; whale oil was found to depress the levels but is unpalatable except when hydrogenated and it then loses its effect. The diets were effective in almost all cases of essential hypercholesteremia and 14 have been kept on a corn-oil diet for a year. It is not possible to assess any change in atheromatosis or in tendon xanthomata.

The general conclusion is that "the cholesterol-depressing effect of certain fats is related to their content of polyunsaturated fatty acids." F. E. HYTTEN

The following remarkable study is one of impeccable design; it provides important data supporting the cholesterol-lowering action of unsaturated fatty acids derived from food sources.

The Influence of Dietary Fats on Serum-lipid Levels in Man. E. H. Ahrens, J. Hirsch, W. Insull, T. T. Tsaltas, R. Bloomstrand, and M. L. Peterson. *Lancet* 1: 943, 1957.

The results are presented of feeding experiments on 40 adults over a period of 4 to 36 months, almost all conducted under strict metabolic-ward conditions. The subjects were either hypercholesteremic or hyperlipemic (some with clinical arteriosclerosis) or normocholesteremic with arteriosclerotic heart disease. All were ambulatory and none were suffering from any condition which was judged to affect long term metabolic studies.

At the beginning of the experiment the patients ate *ad libitum* until relatively constant serum lipid levels were achieved. They were then transferred to formula feeding on corn oil or coconut oil fat providing 40 per cent of the calories. On corn oil the serum lipid levels invariably fell to a steady low level over a period of about three weeks. They rose again on coconut oil.

The fall in lipid level on the corn-oil diet showed a marked individual variation and was not closely related to the initial level. A number of experiments in which other fats were substituted isocalorically for corn oil are described and analyzed in considerable detail. All caused a rise in serum lipid above the level characteristic of the corn-oil diet. The rises varied with the type of fat used and the only conclusion which could be reached was that the effect on serum cholesterol and phospholipid level is strongly correlated with the iodine value of the dietary fat. Three feeding tests using partially hydrogenated oils suggested that raising the iodine number of a fat enhances its action in raising serum-lipid levels.

The highest serum lipid levels were caused by the feeding of butter and coconut oil. While these fats were the most saturated they were also the only fats containing a high content of short-chain fatty acids. Experiments comparing the effects of butter and cocoa butter suggested that fatty acids of short or intermediate chain length cause higher lipid levels in the serum than do long chain saturated acids. This paper is presented and discussed at great length and in considerable detail; it is essential reading for workers in this field. F. E. HYTTEN

A healthy situation exists in medical topics when complete and unanimous agreement does not exist. The following report differs in its conclusions from those above.

Effect of Long-chain Polyunsaturated and Saturated Fatty Acids on the Serum-lipids of Man. L. Horlick and B. M. Craig. *Lancet* 2: 566, 1957.

Yet another report of the effect of dietary fats on blood lipids. In this experiment male medical students and interns, performing their regular duties were given isocaloric diets, each for a period of one to three weeks and each containing a different source of fat, except for a control period of *ad lib* feeding, and in some cases a low-fat (4 per cent) diet.

The serum-cholesterol estimated on fasting serum was depressed equally by removing animal fat from the diet, or by substituting corn oil or ethyl linoleate. Ethyl stearate, of which about 70 per cent was shown to have been absorbed from amounts of 70 g daily did not raise the serum cholesterol after a period of feeding with ethyl in linoleate or after a low fat diet, although there was an increased percentage of cholesterol carried as β-lipoprotein. These results do not support the hypothesis that highly unsaturated fatty acids specifically depress the serum cholesterol. F. E. HYTTEN

Idiopathic hyperlipemia is a condition about which little is known at present. If the following observations are confirmed a means for further studies of the disease will be provided which should furnish clues concerning its pathogenesis.

Effects of Unsaturated Fat on Serum Lipids in Idiopathic Hyperlipemia. M. A. Everett, W. D. Block,

F. A. J. Kingery, and A. C. Curtis. *Proc. Soc. Exper. Biol. & Med.* 95: 500, 1957.

Two patients with idiopathic hyperlipemia were placed on carefully controlled diets. After a control *ad lib* fat intake, the diet included 120 g fat containing 50 g highly purified soybean oil, 30 g soy phospholipid concentration, and 40 g animal fat. Other variations were also administered. Total caloric intake was 2,400 to 2,600. The results indicate a marked decrease in serum lipids, which was independent of the dietary phospholipid concentration. Unsaturated fatty acids in the dietary fat was believed to be responsible for the serum changes. With the decrease in serum lipids, there was an improvement in the clinical status of the patients.

S. O. W.

The new technic for investigation of atherogenesis in tissue culture introduced by Rutstein promises to afford very significant information in this and related fields.

Effects of Linolenic and Stearic Acids on Cholesterol-Induced Lipoid Deposition in Human Aortic Cells in Tissue-Culture. D. D. Rutstein, E. F. Ingenito, J. M. Craig, and M. Martinelli. *Lancet* 1: 545, 1958.

In this well and profusely illustrated paper the deposition of lipoid within human aortic cells in tissue culture is studied.

The culture medium was 40 per cent human blood serum, 20 per cent chick-embryo extract, and 40 per cent Hank's solution with penicillin and streptomycin. When cholesterol in ethanol was added to the medium at a concentration of 1 mg of added cholesterol/100 ml there was little evidence of intracellular lipoid deposition but it increased as the concentration increased and was considerable at a concentration of 5 mg/100 ml with greatly distended cells. A similar result followed the addition of cholesterol bound to beta-lipoprotein.

The deposition caused by the added cholesterol was completely inhibited by the simultaneous addition of linolenic acid (1 mg/100 ml) and was increased by the simultaneous addition of stearic acid (1 mg/100 ml).

F. E. Hytten

Essential Fatty Acids and Idiopathic Hypercalcaemia of Infancy. A. T. James, J. Webb, T. Stapleton, and W. B. Macdonald. *Lancet* 1: 502, 1958.

Idiopathic hypercalcemia of infancy seems particularly to be associated with dried milk feeding and is widely felt to be due to overdosage of vitamin D. However, the suggestion has been made that loss of essential fatty acids from dried milk during processing may be implicated.

The possibility was investigated in three cases. It was first established that the drying process made little difference to the linoleic and linolenic acid content of milk but it diminished very markedly during storage at room temperature. In the three infants the blood levels of linoleic + linolenic and arachidonic acid were normal. When cottonseed oil was given by mouth, there was a transitory fall in the serum calcium level, probably due to an increased fecal excretion of calcium, but it rose again in spite of the cottonseed oil and there was therefore no evidence that a deficiency of essential fatty acids is associated with hypercalcaemia.

F. E. Hytten

Effect of Hydrogenated Triolein on Utilization of Essential Fatty Acids in the Rat. R. B. Alfin-Slater, L. Aftergood, L. Ningemann, G. D. Kryder, and H. J. Deuel, Jr. *Proc. Soc. Exper. Biol. & Med.* 95: 521, 1957.

Weanling male rats were placed on a diet adequate in all respects except fat. After 12 to 16 weeks, the animals were deficient in essential fatty acids, as was indicated by the appearance of scaliness of tail and paws, rough coat and general poor health. After that time, the rats were placed into individual cages for eight weeks on one of the following diets: (1) no supplement, (2) supplements of 250 mg hydrogenated triolein, (3) 500 mg hydrogenated triolein, (4) 20 mg linolate, (5) 250 mg hydrogenated triolein plus 20 mg linolate, (6) 500 mg hydrogenated triolein plus 20 mg linolate, (7) 50 mg linolate, (8) 250 mg hydrogenated triolein plus 50 mg linolate, (9) 500 mg hydrogenated triolein plus 50 mg linolate. The animals fed the linolate supplement alone or in combination with hydrogenated triolein gained weight, and no antimetabolic activity of hydrogenated triolein was noted. M. Silberberg

ITEMS OF GENERAL INTEREST

The Relationship of Leukocyte Alkaline Phosphatase to "Stress," to ACTH, and to Adrenal 17-OH-Corticosteroids. W. N. Valentine, H. Follette, D. H. Solomon, and J. Reynolds. *J. Lab. & Clin. Med.* 49: 723, 1957.

The authors report the results of the determination of serum proteins, lipoproteins, and glycoproteins in patients with idiopathic hypercholesterolemia and idiopathic hyperlipemia.

In idiopathic hyperlipemia there is a lactescent serum in the fasting state and the levels of triglycerides and total lipids are increased. In hypercholesterolemia on the other hand the serum is clear and there is an elevation of cholesterol and phospholipids. Both diseases have an elevation of the beta-lipoprotein fraction as determined by Cohn fractionation.

As shown by ultracentrifugation, the main alteration in the patients with idiopathic hyperlipemia is an elevation of the $\overline{f}$ F 20 to 100 and 100 to 400 lipoproteins while in idiopathic hypercholesterolemia the levels of $\overline{f}$ F 0 to 12 and 12 to 20 lipoproteins are markedly increased. By paper electrophoresis it has been demonstrated that patients with idiopathic hyperlipemia exhibit a marked increase in the O-fraction while those with hypercholesterolemia have a beta-lipoprotein spike.

In this study the authors confirmed previous work and present data to show that patients with idiopathic hyperlipemia have an increase in alpha-2-"glycoprotein" whereas serum "glycoprotein" patterns in idiophathic hypercholesterolemia did not differ from those observed in the controls. They also demonstrated that there was no change in the serum "glycoprotein" levels in healthy controls following a standard fat-loading meal despite elevation of total lipids and an increase in the O-fraction of the lipoproteins.

K. R. CRISPELL

Two Forms of Necrotizing Arteritis in Dogs Related to Diet and Renal Insufficiency. H. C. McGill, J. C. Greer, J. P. Strong, and R. L. Holman. *A. M. A. Arch. Path.* 65: 66, 1958.

This investigation tries to establish morphologic differences in the arteritis produced by renal insufficiency alone, and by a combination of renal insufficiency with feeding of a high fat diet. Mongrel dogs weighing 2 to 5 kg were fed a diet low in protein and in which 43 per cent of the calories were derived from butter fat. This diet was fed in amounts of 75 cal/kg body weight for about two months. It produced a weight increase of about 10 per cent above that of control dogs fed a stock diet. Subsequently, renal insufficiency was produced by subcutaneous injection of 8 mg/kg body weight of uranium nitrate in aqueous solution, and if this dose was insufficient, of a second injection of 16 mg/kg body weight of uranium. Tubular kidney disease resulted. In other dogs bilateral nephrectomy was performed. Eighty per cent of the animals fed the high-fat diet developed changes in the large mucoelastic arteries. These lesions were characterized by changes in the glycoproteins of the ground substance as indicated by appearance of metachromasia. This "muco" arteritis was seen with equal frequency in nephrotic as well as nephrectomized dogs. The dogs fed the stock diet showed lesions referred to as "myo" arteritis. These lesions consisted primarily of necrosis of the smooth muscle of muscular arteries and arterioles, and they were much less frequent in animals with uranium nephrosis than in nephrectomized animals.

M. SILBERBERG

Polycyclic Aromatic Hydrocarbons in Coffee Soots. M. Kuratsuno and W. C. Hueper. *J. Nat. Cancer Inst.* 20: 37, 1958.

Two different types of coffee soots obtained during different roasting processes from two plants in the Southern United States were collected from the bottom stacks attached to the roasters. The caffeine content was about 17 per cent. The soots were ground to a fine powder and dissolved in benzene or acetone. The extracts were analyzed chemically and by chromatography. In the benzene but not in the acetone eluates ten different polycyclic aromatic hydrocarbons were identified, amongst them benzo (a) pyrene which is known to be a carcinogenic agent in experimental animals. These findings do, however, at the present not permit definite conclusions as to the carcinogenicity of coffee soots in humans.

M. SILBERBERG

Effects of Brief Exposures to Cold on Performance and Food Intake. B. Weiss. *Science* 127: 467, 1958.

Experiments on rats, based on brief exposures to varied temperatures, demonstrate an acclimatization phenomena of very short latency. This was based on an experimental design in which the frequency of pressing a bar to deliver food could be measured in relation to the environmental temperature. It appears that day-to-day regulation is quite sensitive to changes in temperature. There is a short latency of response to these effects.

S. O. W.

Basal Metabolic Rate in Old Men. J. D. Robertson. *Lancet* 1: 296, 1958.

Data on basal metabolic rates in the very old are extremely rare in the literature. In this study five healthy, normally active men between 91 and 100 years old were tested.

The mean heat production was 29.5 cal/square meter/hour (range 27.7 to 31.8), somewhat lower than that predicted by extrapolation of younger age data.

F. E. HYTTEN

Gastric Function in Pregnancy. J. N. Hunt and F. A. Murray. *J. Obst. & Gynaec. Brit. Emp.* 65: 78, 1958.

The common pregnancy complaint of heartburn has sometimes been blamed on delayed gastric emptying but there is very little information.

In this investigation seven normal pregnant women were studied by means of saline and water test meals throughout pregnancy. In so far as these tests indicate everyday gastric function, there was no slowing of emptying; possibly a slight reduction in time occurred in the later weeks. The secretion of acid, chloride, and pepsin was reduced and reached their lowest values at about the 30th week.

Lactation was associated with a marked increase in gastric secretion which did not occur in women who were not breast feeding.

(Since enzyme secretion is reduced, digestion may be slowed and gastric emptying after an ordinary meal correspondingly affected. Deductions drawn on the basis of saline test meals would then be of little practical help.)

F. E. HYTTEN

Hyperpyruvaturia Following Intravenous Administration of Fructose. V. Hoenig. *Lancet* 1: 506, 1958.

The fact that fructose is more readily assimilated and metabolized by diabetic subjects has led to a number of investigations on other differences in the body's handling of the two sugars.

In the present investigation 25 g of glucose or fructose in 40 per cent solution was given intravenously to a number of normal persons and patients with cirrhosis of

the liver. In both groups fructose caused a very pronounced increase in the excretion of urinary pyruvate: (about 118 μg/min per 1.73 sq meter of body surface, compared to a usual value of about 3 μg) which disappeared within an hour of the administration of the fructose.

The fructose apparently interferes with the metabolism of the renal tubular cells, depressing the reabsorption of pyruvate. F. E. HYTTEN

Effects of Beta-Aminopropionitrile Upon Wound Healing. G. A. Krikos and J. L. Orbison. *A.M.A. Arch. Path.* 65: 312, 1958.

Weanling rats of the Wistar strain and adult rats weighing 284 to 440 g were fed a diet of Purina Fox Chow supplemented with 0.4 per cent β-aminopropionitrile fumarate. Animals receiving the stock diet only served as controls. After nine days of observation, a circular wound in the skin was made, measuring 1 cm in diameter, and the course of wound repair was studied over periods up to 21 days. In animals receiving the diet containing the lathyrus factor epithelization of the wound defects was not affected at all. However, the lathyrus factor interfered with the development of the granulation tissue in the wound base. The granulation tissue was less cellular and contained fewer collagenous fibers than in the controls. Consequently, there was inhibition of contraction of the wound margins prior to closure. These effects were more marked in young than in adult rats. The results of this investigation represent another example of the harmful effect of lathyrism on the mesenchymal structures, which has already been demonstrated in bone and vessels. M. SILBERBERG

Effects of Ethionine Administration in Rabbits and Dogs. I. Changes in Serum Proteins, Lipids, Lipoproteins, and Glycoproteins and in Blood Coagulation. C. Wang, F. Paronetto, E. Sohars, and D. Adlersberg. *A.M.A. Arch. Path.* 65: 279, 1958.

Male and female gray chinchilla rabbits each weighing about 3.3 kg received daily intraperitoneal injections of a 2.5 per cent solution of ethionine in saline varying from 100 to 500 mg/day for periods up to 10 days. Anorexia, loss of weight, and emaciation resulted. The toxic effects were more marked in females than in males. High doses led to a temporary increase of serum lipids and lipemia; medium high doses caused a mild decrease of serum lipids followed by slight lipemia; small doses depressed lipids to low levels. Irrespective of the dose, the blood sugar values were decreased, but serum amylase rose slightly. Total serum proteins decreased as shown by both chemical analysis and paper electrophoresis. The relative amount of albumin increased, while beta- and gamma globulins were slightly decreased. Serum lipoproteins and glycoproteins decreased markedly. Blood coagulation and prothrombin time were prolonged. Similar studies were carried out in male and female dogs each weighing 5.7 to 15 kg and having received 100 mg/kg of a 2.5 per cent solution of ethionine every other day for periods up to 17 days. The urine of these dogs contained bilirubin, and the sera became icteric. The remaining findings were on the whole similar to those observed in rabbits except that blood sugar and amylase were not affected. M. SILBERBERG

Effects of Ethionine Administration in Rabbits and Dogs. II. Pathological Studies. C. Wang, L. Strauss, F. Paronetto, and D. Adlersberg. *A.M.A. Arch. Path.* 65: 286, 1958.

The tissue changes seen in rabbits and dogs treated with large amounts of ethionine are described. The liver showed hemorrhages, fatty change, or focal necrosis. The higher the dose of ethionine, the more severe the liver damage. The lungs and gastrointestinal tract showed petechial hemorrhages probably due to the decrease of platelets and the disturbed clotting mechanisms. In advanced cases gastric and duodenal ulcers were seen. In the pancreas, the acinar cells were swollen, vacuolated, and depleted of zymogen granules. If damage was severe, the acinar structure became obliterated, and hemorrhages and fat necrosis with calcification and foreign body reaction resulted. The latter changes correlated with the rise of serum amylase but not with the degree of lipemia. In the spleen, the Malpighian bodies were decreased in size, and hemosiderin was markedly increased apparently due to increased blood destruction. The adrenal cortices, in particular the zona glomerulosa and fasciculata were hypertrophic, the cells being loaded with fat. These changes correlated with the rise in serum lipids. However, there was also lipemia without morphologic change in the adrenals. The tubular epithelia of the kidneys and the myocardial fibrils underwent fatty changes. Rabbits were more susceptible to pancreatic lesions than dogs, while the gastrointestinal changes were more often seen in dogs than in rabbits. If the treatment with ethionine was discontinued, repair often took place. M. SILBERBERG

Some Considerations Pertaining to the Proper Use of Supplementary Vitamins. W. J. Darby. *J. Chron. Dis.* 6: 2, 1957.

"This presentation attempts to state the author's opinion as to the major considerations bearing on the use of vitamins." He categorizes the therapeutic use of vitamins into three classes: (1) The curative treatment with specific administration of factors to correct an evident deficiency state; (2) protective treatment or the prevention of the development of deficiency states in individuals who are especially exposed to the possibility of a deficiency; (3) preventive supplementation designed to offer widespread general prevention in the population as a whole and includes the addition of specific nutrients to foods.

The magnitude of any increased needs of vitamins due to disease is a point on which there is little objective information. The author points out that depletion of

moderately well nourished adults requires a variable period of from weeks to years. Useful chemical methods are available to assist in ascertaining when the patient's stores of several vitamins have been replenished and, when further supplementation, is, therefore, unnecessary. He recommends as a guide to vitamin supplementation the Recommended Daily Dietary Allowances for Public Health Hospitals.

This is a timely review and again stresses the fact that an appropriately varied diet supplies a sufficient quantity of those nutrients required by man to meet his requirements except under unusual circumstances.

K. R. CRISPELL

Influence of Heating on Artificially Induced Antibacterial Agglutinins in Milk. V. W. Greene, J. C. Olson, and J. J. Jezeski. *J. Dairy Science* 40: 1250, 1957.

This is a phase of the researches being conducted at the University of Minnesota where it has been demonstrated that specific antibodies can be introduced into cow's milk by infusing the udder with killed suspensions of pathogenic micro-organisms. In this study agglutination tests were applied to the acid wheys of milks of cows subjected to the infusion technic; in this case using a phenol-killed suspension of *Salmonella pullorum* cells. No significant loss of antibody potency was observed on exposing the milk to moderate temperatures such as would be employed in pasteurizing and drying milk, 155°–165° F, for periods less than 25 min.

F. E. RICE

An Antibody to Castle's Intrinsic Factor. K. B. Taylor and J. A. Morton. *Lancet* 1: 30, 1958.

Precipitin-type antibodies were detected in rabbits injected with adjuvant-treated preparations of human and pig intrinsic factor (I.F.). Precipitin (agar gel diffusion) tests of intrinsic factor antigens and appropriate antisera gave heavy precipitates. There was cross-reactivity between pig I.F. and rabbit anti-human-I.F. serum. None of the antigens were pure I.F., but the antisera prepared to them were able to neutralize I.F. activity in vitro. Neutralization of I.F. activity was determined in five pernicious anemia patients by the percentage absorbed of an 0.5 μg dose of Co^{60}-labeled vitamin B_{12}. In two of the patients there was 100 per cent reduction of absorption of the labeled B_{12}. Agar gel (Ouchterlony plate) immuno-chemical procedures used may prove of further use in testing the homogeneity and purity of I.F. preparations. The authors pose the question of whether or not parenteral I.F. is essentially a foreign antigen due to partial or complete species specificity. If rabbits can produce circulating antibodies to I.F., can man? Newer methods for the detection of antibodies in serum and other body fluids should provide an answer in the near future.

E. COHEN

Dietary Production of Lipogranuloma in Rats. A. J. Cox, Jr. and F. de Eds. *Am. J. Path.* 34: 263, 1958.

Male and female rats received isocaloric rations of a base diet supplemented with 5, 10, or 20 per cent acetoglycerides. The protein content was kept constant at 17.6 per cent. The animals were observed for periods up to 700 days. As early as after 30 days, but increasingly with advancing age, the fat tissue particularly about stomach, intestine, pancreas, and sex organs showed granulomatous lesions. The latter consisted of foam cells, foreign body giant cells about crystal deposits and mild subacute or chronic inflammatory reaction. These lesions seem to be due specifically to acetostearines since supplements of 50 per cent of butter or tallow to the diet failed to produce such lipogranulomas. Reinforcement of the acetostearin-enriched diet with vitamin E did not prevent the development of these lesions. The lipogranulomas were similar to those seen in human infantile fat necrosis, or so-called sclerema adiposum neonatorum. Although there is as yet no definite evidence that lipogranulomatosis of humans is related to diet, this problem seems worthwhile investigating in view of the above results.

M. SILBERBERG

Purification of Intrinsic Factor. L. Ellenbogen, S. H. Burson, and W. L. Williams. *Proc. Soc. Exper. Biol. & Med.* 97: 760, 1958.

A highly potent intrinsic factor preparation was obtained from hog duodenum by saline extraction, pH adjustment, and ammonium sulfate precipitation. Five mg daily induced a remission in a patient with pernicious anemia in relapse. The preparation was five times as potent as the U. S. P. intrinsic factor reference standard in increasing the urinary excretion of vitamin B_{12} in patients with pernicious anemia in remission. Electrophoresis and utracentrifugation showed that it was not homogeneous. Complete separation of the components by ultracentrifugation was not possible. Passage through a cellulose ion exchanger gave a fraction which was five times as potent as the original preparation but which was still not homogeneous on ultracentrifugation.

G. WALKER

Food Utilisation and Weight Gain in Hypophysectomised Rat as Function of Handling. H. Elrick and L. Bernstein. *Proc. Soc. Exper. Biol. & Med.* 97: 903, 1958.

Male Sprague-Dawley rats were hypophysectomized when 19 days old and were allowed food *ad libitum*. Half the animals were picked up and stroked for 10 minutes daily for six weeks and the remainder were not touched at any time during the experiment. The animals which were handled ate less food but gained more weight ($p = 0.05$ in each case) and thus utilized their food more efficiently than the non-handled controls. There was no difference in the rate of skeletal growth or in the proportion of protein and fat in the carcasses of the two groups of animals.

G. WALKER